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EPIDEMIOLOGICAL STUDIES

Ralph E. Wheeler, et al

Public Health Service
Cincinnati, Ohio

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EPIDEMIOLOGICAL STUDIES

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Supplement "E" to Final Report to the Ohio River Committee Ohio River Pollution Survey

1798

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SUMMARY

Field studies of water-borne disease on the Ohio River watershed were made during the Ohio River Pollution Survey, 1939-1940. These studies covered field epidemiological investigation of outbreaks of apparently water-borne disease; field bacteriological studies of these outbreaks; laboratory experimental studies, not definitely connected with outbreaks of disease, and statistical study of mortality from diarrheal and enteric diseases throughout the watershed.

Two outbreaks of gastro-enteritis, a mild affliction characterized by nausea, vomiting, cramps and diarrhea, lasting but a few days and, practically with no mortality, were studied in detail.

One outbreak of bacillary dysentery (Shiga), an acute illness, extending over several weeks with considerable mortality, was studied during the closing days of the Survey. This outbreak of dysentery contrasts sharply with those of the mild, transitory gastro-enteritis.

Evidence was obtained that this type of gastro-enteritis is of infectious nature and water-borne. Experimental studies on human volunteers suggested the possibility that this form of gastro-enteritis may be due to some hitherto unrecognized causal organism which may not be detected by the usual bacteriological tests applied to determine the potability of water.

Statistical evidence obtained in 43 cities of the Ohio River watershed, indicate that filtration and chlorination of water supplies did not reduce the diarrhea and enteritis death rate to the same extent that these measures reduced the typhoid fever death rate, basing this finding on a comparison of death rates for the 5-year period preceding the establishment of filtration and/or chlorination with the 5-year period immediately following installation of these water purification procedures.

WATER-BORNE DISEASE STUDY

I. Aims

The virtual conquest of water-borne typhoid fever along the Ohio River has been effected by the installation of adequate water treatment facilities in the great majority of the cities and towns using surface water supplies. There remains, however, an epidemic disease (by no means limited to the Ohio River Valley) generally considered to be carried by water and usually referred to as "Gastro-enteritis, presumably water-borne". Individual cases of this ailment show symptoms of nausea, vomiting, cramps and diarrhea lasting usually one to three days. So similar are the symptoms to the common food-intoxications that patients are quite likely to attribute their trouble to something eaten at a recent meal, until they learn of numerous cases occurring simultaneously among friends and neighbors. Because food poisoning is a relatively common occurrence and the symptoms, though often severe, have been experienced before, a physician is not likely to be called and extensive epidemics with cases occurring actually in the families of physicians have escaped medical attention entirely or have been dismissed as unimportant. Individual cases are not always easily distinguished from summer diarrhea or mild dysentery. The vomiting, the lack of fever, the short course, the watery, often fetid, stool and the aversion to fluids despite often severe dehydration, are more typical of gastro-enteritis than of the above mentioned more ominous conditions.

Although individual cases are not always easily differentiated from the more common ailments noted above, the disease is easily identified by its behavior in groups of people--in other words by its epidemiology. When the specific cause of a malady is unknown, it not infrequently happens that it can be more readily identified by its mass behavior than by the symptoms produced in individual cases. Thus influenza was formerly differentiated from grippe and other seasonal disturbances by its high attack rate and rapid spread from person to person. Water-borne gastro-enteritis--often erroneously diagnosed "intestinal influenza"--shows these same attributes in even more striking degree. As much as 80 percent of the population of a town has been known to be attacked within a period of two or three days to a week--a far more explosive condition even than influenza, which usually requires a full week, even in a small community, to reach its maximum prevalence and is not wholly over in two or three weeks. Like influenza, this form of gastro-enteritis is frequently a cold weather disorder and all ages are indiscriminately attacked. These characteristics assist in differentiating the condition from the food poisonings and summer complaints which tend to prevail in summer and early fall and attack the younger age-groups more than the older. Quite striking, also, is the low mortality from water-borne gastro-enteritis when this is not accompanied by another more lethal water-borne condition such as typhoid fever. Less than one person dies per thousand persons attacked and the occasional death usually occurs in individuals stricken with some co-existing acute or chronic disease--almost never in a previously healthy person.

As was noted above, the cause of true water-borne gastro-enteritis is obscure. Occasionally outbreaks have been explained on a chemical or specific bacteriological basis. Because the human gastro-intestinal tract has only a limited number of responses to a wide variety of harmful materials, it is not impossible that each outbreak may have a separate cause. It is known that the drinking of water contaminated with relatively small amounts of sewage can result in acute gastro-enteritis. Outbreaks of gastro-enteritis from this cause occurred more frequently two or three decades ago than they do at present, and were often followed by infections with the typhoid organism, then fairly common in sewage.

Sewage regularly contains great numbers of coliform bacteria, in and of themselves harmless, but valuable as indices of sewage contamination in drinking water. Tests for the detection of coliform bacteria are therefore made as a routine procedure in most water treatment plants.

Occasional outbreaks of gastro-enteritis have been traced, however, to water in which sewage pollution was not found by the coliform tests applied. The impression has crystallized among leaders in the water treatment field that causes other than sewage pollution may occasionally produce gastro-enteritis, and it was thought advisable to make a determined effort to learn the cause or causes of these outbreaks, so that, if really needed, further tests could be devised to insure the safety of drinking water.

Moreover, there was another cause for official concern. Physicians in attendance upon cases of summer complaint and the various diarrheal diseases of the warm months, puzzled by the almost epidemic prevalence of these conditions at times, not infrequently blamed the water supply either for inadequate treatment or for excess of chemicals.

The problem appeared therefore to be a two-fold one--first, to determine the causes and prophylaxis of water-borne gastro-enteritis of hitherto undetermined etiology, and second, to learn enough, if possible, of the causes and mode of infection of summer complaints to determine their relationship to water as a possible vehicle of the ailment.

II. Personnel and Equipment

To fulfil this dual purpose the epidemiological unit was organized and equipped for a somewhat diversified attack on the problem by the late Surgeon Filip C. Forsbeck. His untimely death in July, 1940, was a serious set-back for the study; arrangements were, however, made to complete the study very nearly in accordance with his original plans. The personnel consisted of an epidemiologist in charge of the unit, who was to carry out field studies on outbreaks and to supervise the work of other members of the unit, and a bacteriologist to examine water, stool, and other specimens secured in the course of the field work. For the preparation of media and glassware a laboratory assistant was assigned to the bacteriologist. Finally there was a statistician for the larger cities of the Ohio River Valley, to assist in the analysis of epidemic data and to help in epidemiological studies in the field during the course of outbreaks.

The headquarters of the unit, which was a subdivision of the Ohio River Pollution Survey, were at the U. S. Public Health Service's Stream Pollution Investigations Station in Cincinnati where extensive laboratory facilities and able advisory assistance were made available. Because it was recognized that water and stool specimens were best examined immediately, a trailer laboratory completely equipped for bacteriological study of pertinent specimens and samples was developed for work at the site of an outbreak.

Good relations with the Health Departments of the several states forming the Ohio River watershed were established early in the study and renewed by periodic collaboration on various projects. Outbreaks of waterborne gastro-enteritis are not common and, because of their ephemeral character, it was necessary to keep in close touch with State health workers--particularly with the sanitary engineering groups, in order to learn of outbreaks during their early phases.

III. Methods of Field Study

When notification of a city or town outbreak was received from a State health department, the local health department having jurisdiction was contacted and permission obtained for participating in the study.

The epidemiologist and statistical assistant usually preceded the laboratory, selected a central location for the trailer and arranged for the electric and other connections which it required.

Definite establishment of the fact that an epidemic was occurring was an early and vital feature of their work. This entailed (1) a study of school absences--which invariably show a sharp increase when an outbreak of any magnitude has begun--and (2) visits to local druggists to inquire whether sales of castor oil, bismuth preparations and paregoric had shown a sudden sharp increase. When these inquires to druggists are properly addressed, surprisingly uniform and frank responses are nearly always obtained. Druggists know the volume of their usual seasonal sales of these preparations and can give surprisingly good statements of the comparative sales during any period of inquiry. The files of recent local newspapers also give good data on the time at which the outbreak, if any, first came to public notice and on the antecedent local weather conditions. If, from these various preliminary inquiries, there was good basis for believing that an outbreak was in progress, a map of the local water distribution system was secured and a number of water samples was obtained from selected sampling points on the main grid of pipes, on all dead ends, and from the source of supply both before and after treatment, if any treatment was used. Besides subjecting these samples to bacteriological study, an important test made on these early samples (where chlorine was routinely used) was a test for residual chlorine.

Next, a house-to-house canvass was conducted of a random sample of the population, using the form shown in appendix (I). This form was developed with a view to obtaining the most important information within a minimum of time. It can be filled in completely in less than 10 minutes in the average household, and two canvassers working for half a day can secure a very respectable population sample. Preliminary studies of this half-day's work usually give a fairly definite idea of whether water is the vehicle of the infection. In canvassing, an attempt was made first to secure a random sample of households straight across the town or city in one direction and then in another direction perpendicular to it. In this way geographic distribution of cases can be added to the information obtained from the age, sex, and other data of importance. In most localities a certain number of persons can be found who do not habitually use the city, or town, water supply for drinking purposes but resort, instead, to well, cistern or bottled spring water. As large a sample of these individuals as possible was obtained early in order to determine the extent to which they were attacked. It is also important, where infants are living in a household, to determine whether their water and milk were boiled or given raw.

An intensive bacteriological study of the water supply was carried on coincidently with the epidemiological survey. In selecting sampling points, a careful inspection was made of the pumping station, treatment plant and distribution system. On the distribution system points for sampling were selected so that freshly treated water would be tested as well as water from dead ends. To obtain more complete information concerning the bacterial content of the samples, two procedures were followed, (1) the sample was examined for coliform bacteria in accordance with Standard Methods to determine whether the water met the requirements of the Treasury Department Standard, and (2) lactose broth tubes showing growth, but no gas, were examined to determine the type of bacteria present. In making determinations on portions of water, much larger in volume than prescribed by Standard Methods, a concentration procedure was developed.

The samples were examined as soon as received. Portions of 10 ml. each were measured into 10 tubes of lactose broth, incubated at 37° C. for 24 hours and examined. A second reading was also made after 48 hours. Those tubes which showed gas were transferred to brilliant green bile broth and streaked to MacConkey's agar plates at each examination period. A positive result in either medium was considered sufficient confirmation for the presence of members of the coliform group.

The tubes which showed no gas but did show the presence of growth were streaked on MacConkey's or other selective media plates, as the nature of the work might indicate, and the plates incubated 18 to 24 hours at 37° C. To obtain information regarding the flora, other than coliforms, present in the water, at least two representative non-lactose fermenting type colonies would be fished from each MacConkey plate to Russell slants. Russell's medium was used because it was considered worthwhile to learn something about the presence of proteus and the atypical slow lactose fermenting types which might be present. If the Russell tubes gave evidence of a consistent flora for the sample, one of the duplicate cultures was discarded at this point and the remaining cultures purified by streaking on MacConkey's agar, after short-time incubation in broth. Two well-isolated colonies were picked from the plate and again inoculated in Russell tubes. Where more than one type of colony appeared, two of each were fished. If these Russell tubes gave similar reactions, one culture only was retained for further study.

For the study of unfinished or raw waters the above procedure was modified as follows: If not highly polluted, five 10 ml. portions were planted into lactose broth directly and three portions in each of several serial dilutions added as indicated. It was always considered essential to have at least one tube in the highest dilution which showed no growth. In all cases primary consideration was given to flora found in tubes with no gas formation or in the dilutions higher than that required to give the coliform index.

Because of the small number of bacteria to be found in treated water, a method of concentration was worked out and given practical test on water samples collected from Cincinnati and the surrounding communities within a radius of 75 miles. By this technique any desired portion of the water sample is filtered, with the use of suction, through an apparatus sold commercially under the name of Jenkins filter. By experimenting it was found that if the original filter blocks supplied with the apparatus were cut to a thickness of approximately 3 to 5 mm., the filtration time for a 100 ml. portion would be about 30 minutes.

Control tests were run on water samples known to be contaminated and on known suspensions of coliforms in distilled water. If the filter disc did not retain greater than 95 percent of the organisms it was discarded. With but few exceptions the filtrates were sterile. If there were many bacteria or an increased amount of inorganic material in the sample, difficulties were met in filtering. In the experimental work two portions of 100 ml. each were filtered. The rubber sleeve holding the filter disc containing the bacteria was removed from the apparatus and with sterile rod the disc was pushed into a tube of enriched medium. Lactose broth and selenite F were used for enrichment. Incubation was at 37° C. and after growth appeared, the material was streaked on MacConkey or other selective media plates, the colonies fished and purified, with the technique outlined above. A comparison of the results of the concentration technique against the results from the ten 10 ml. portions planted in the standard manner showed a close agreement in the number of coliforms found. Moreover the same strains of bacteria were isolated by the concentration method as were obtained with the standard procedure.

After the cultures obtained by the above described methods had been purified, they were examined for gram reaction. Impure cultures were replated. Cultural, physiological, and fermentation characters were obtained and a tentative identification made. It was found that with these methods it was frequently possible to obtain organisms belonging to the genera Salmonella, Eberthella, Shigella, Pseudomonas, Proteus and Alcaligenes as well as atypical coliforms. It is not clear what relationship these forms bear to the pollution of the water. After a careful study of many strains which appeared to be Salmonella, Eberthella or Shigella the results indicated that they were in reality atypical forms of Proteus. There were many forms which we were unable to identify by our present system of classification. Dr. P. R. Edwards of the Department of Animal Pathology, Agriculture Experiment Station, University of Kentucky kindly assisted with the identification of the suspected Salmonella strains. In one instance a culture of S. aertrycke was isolated from a dead end sample taken from a Cincinnati water tap. On several occasions S. oranienburg was isolated from raw water of Royal Spring, the source of supply for Georgetown, Kentucky.

Cultures which gave a neutral reaction on Russell's double sugar or Krumwiede's triple sugar tubes were at first discarded as being Alcaligenes and unimportant. Later work indicated that these cultures were frequently a variety of Pseudomonas. It was found that very often cultures of Pseudomonas were present which failed to give the fluorescing character used for identification. Special methods were devised for identifying these strains but this work as yet is incomplete. All cultures which gave neutral or alkaline reactions on Russell's or Krumwiede's were sub-cultured on synthetic dextrose agar slants. Pseudomonas on this medium gives an acid slope with a neutral butt. Most cultures produce a fluorescence when grown in synthetic asparagin broth containing mannitol. Thus far all of these strains have been citrate positive and indole negative.

In epidemics of this type it is of major interest to find out whether any of the organisms present in the water supply appeared as the predominant organism or in any considerable number in the stools of gastro-enteritis cases which had used the supply. Where possible stool specimens were obtained and examined microscopically and smeared on plates of selective media such as MacConkey's agar, desoxycholate citrate agar, Bacto-Shigella-Salmonella agar and bismuth sulfite agar. Suitable portions of the stool were placed in selenite F for enrichment and the growth streaked on the selective media. Suspicious colonies were fished to Krumwiede's triple sugar tubes and those showing a neutral reaction throughout, or a neutral slant and acid butt, with or without gas, were further identified by cultural, physiological and serological characters.

Small outbreaks involving a limited number of persons, or families, drinking water from a polluted well or cistern, or involving some institution on a private supply are probably not uncommon. These would not ordinarily come to the attention of the state health departments.

IV. Findings in Water-borne Gastro-Enteritis Outbreaks

Three outbreaks were brought to the attention of the Epidemiologic Study Unit during the period of active field investigation.

1) The first occurred in an Indiana town of nearly 9,000 persons early in February, 1940. The Unit was first informed of the outbreak several months afterward but detailed studies, made by the Bureau of Sanitary Engineering of the Indiana State Board of Health and summarized in a Bureau Report of April 29, 1940, were kindly submitted to the unit. A house-to-house canvass of 1,431 persons in the town, soon after the epidemic, showed that 302, or 21 percent, had suffered with nausea, vomiting or diarrhea between February 1st. and February 5th. The figure 1,431 includes 271 persons obtaining their drinking water from wells, cisterns and other independent sources. As none of these latter persons reported an attack, the incidence rate for the remainder, using town water, was nearly 26 percent. This locality obtains water from a river, at a point, thirty miles downstream from a town of 10,000 inhabitants. A prolonged cold spell had converted the river at low stage into an ice-covered conduit. Toward the end of January several million gallons of strawboard-waste had broken through an embankment into the river above both towns, suddenly and tremendously increasing the chlorine demand and contributing undesirable tastes and odors. Chlorine dosage was not increased sufficiently to cover the chlorine demand of the water in the town experiencing the outbreak, and repairs to filters were in progress, permitting turbid water to get into the mains on more than one occasion. A probable explanation, then, was that nearly untreated sewage-polluted water was used for drinking.

Other towns using surface water still further downstream suffered progressively milder outbreaks at successively later dates, but towns supplied from wells escaped.

This series of outbreaks suggests a small-scale repetition of the series of outbreaks occurring on the Ohio River in the winter of 1930-31 and described by Veldee (1).

2) On November 18, 1940, the Division of Sanitary Engineering of the Kentucky State Health Department invited the collaboration of the water-borne disease unit in the study of an outbreak characterized by nausea, vomiting, cramps and diarrhea in the city of Georgetown, Kentucky. A detailed report of this outbreak is contained in appendix(II). This epidemic was studied early, under very nearly ideal conditions of observation, and revealed an epidemiological pattern with which other outbreaks observed in the study and culled from the literature have conformed.
First, the onset of the epidemic was explosive and the duration short.
Second, the incidence among persons using private well and cistern water for drinking was much lower than that among persons using the city supply.
Third, the age and sex distribution of cases in a canvassed population was nearly uniform, indicating that there is no increment of immunity in a population with age or occupation.
Fourth, there was a definite incubation period of 36-72 hours, suggesting an infectious, as distinct from a toxic, etiology.
Fifth, explosive gas formation in standard lactose broth was noted in the 48 hour reading of numerous water samples taken early in the course of the outbreak but this, with the exception of one sample, failed to confirm for coliforms.
Sixth, stools of patients, extensively studied with reliable media, failed to show a common etiological agent.

In detailed studies of all but one of the early water samples and from a limited number of stool specimens, organisms identified as a species of Pseudomonas were isolated and hopes were entertained of this being shown as a causal factor. However, more extensive experimental study of three human volunteers with this and other strains did not confirm these hopes. The finding, however, warrants mention as no other organism was found common to both stool specimens and water samples from this outbreak.

The source of supply, the Royal Spring, was found to be subject to more or less extensive pollution--particularly after rains. Treatment (coagulation, filtration and chlorination) was found to be inadequate. The settling basins were heavily lined with blue-green algal masses (oscillatoria) and dead masses floated on the surface, adding to the organic load and possibly contributing both a tint and a taste to the treated water.

No explanation was found, however, for the comparative freedom from pollution, as evidenced by standard tests of the treated water for coliform bacteria at the time the early samples were taken.

3) Early in March, 1941, the water-borne disease study unit was invited by the Ohio State Health Department to participate in a study of an outbreak closely resembling the Georgetown epidemic, in the village of Navarre, Ohio. A detailed report of this outbreak is contained in appendix(III). As this occurrence was nearly over before it came to official notice, no stool specimens were secured. Early tests of the village water by the State Health Department laboratories revealed the presence of coliforms in 10 ml. and even 1 ml. portions. In other respects, however, the outbreak was nearly an exact replica of the Georgetown occurrence noted above.

No evidence of contact transmission could be obtained in house-to-house canvass. Where some in a given home had used the suspected supply and others had not, the infection was always limited to the former. Prolonged pumping of the wells from which the water supply was derived, showed very limited numbers of organisms appearing only at the end of the pump run. These organisms were identified as a species of Pseudomonas. Coliforms at no time appeared in the samples from the pump, though in samples from some points on the distribution system slow-lactose fermenting organisms and Pseudomonas were found. No evidence of pollution at the source, and no point at which pollution entered the distribution system was definitely discovered in Navarre.

The three outbreaks outlined above were the only ones brought to the attention of the unit. The last two were studied under exceptionally favorable conditions and with considerable care. No definite etiological agent was found. However, certain features were ascertained. The outbreaks at both Georgetown and Navarre were quite conclusively proven to be water-borne. An incubation period for water-borne gastro-enteritis was established, suggesting an infectious etiology. Finally, a study of stool specimens in one typical outbreak failed to show an etiological agent.

V. Literature on Water-borne Gastro-enteritis

A study of previous experience with, and of the literature on, water-borne gastro-enteritis was undertaken with a view to amplifying these rather limited conclusions.

A detailed study of this entity by Cox (2) presents a list of hypotheses on the etiology, then a general consideration of a number of outbreaks, as well as a detailed study of a few, and concludes that pathogenic bacteria are usually involved. With his concept that the disease "is in the nature of a mild dysentery" some exception must be taken for reasons which will appear below.

The ensuing paragraphs will summarize briefly a number of references to outbreaks, a few of which were covered by the article of Cox mentioned above. Some of these were gleaned from the valuable publication of Wolman and Gorman (3) and others from various reference sources, as well as from correspondence with persons familiar with the circumstances. Certain details of these outbreaks not mentioned in the text are given in the table in the Appendix (V) when details were available. These include date and day of week of the first cases; duration of outbreaks; date of collection of water samples and interval in days between first cases and first samples; presence or absence of coliforms in first samples; source of water supply, number of persons using the supply, number and percent attacked and, finally, general remarks on each outbreak. The three outbreaks described in the previous section of this report have also been included in the appendix summary table and will be discussed together with those gathered from the literature.

Although the outbreaks to be described briefly below all bear a strong mutual resemblance in their epidemiological features, they are differentiated into three groups on the basis of certain findings. The first group consists of epidemics of gastro-enteritis, apparently water-borne, in which no conclusive evidence of sewage pollution in the treated water was found by bacteriological analysis, and in which sanitary studies failed to disclose sufficient evidence of pollution of the supply by sewage. The second consists of epidemics with evidence of sewage pollution but without ensuing cases of typhoid fever. The third group consists of epidemics identical with the second except that typhoid fever followed the initial gastro-enteritis outbreak.

Group I. Of the three outbreaks described in the previous section, only that of Georgetown belongs in the first category, and this is possible only because the bulk of evidence indicated surface drainage, rather than sewage, as the most probable means of contamination of the water supply. A search of available sources reveals three others occurring in localities whose water, at the time of testing, met bacteriological standards of potability. These will now be described briefly.

Through the courtesy of the Massachusetts State Department of Health, the records of an outbreak of water-borne gastro-enteritis were made available to the unit. This epidemic occurred in Cohasset, Massachusetts, in March, 1931, and was studied by one of the authors, then epidemiologist in the Massachusetts State Department. Except for the fact that vomiting played a rather more prominent role than cramps or diarrhea, it followed very closely the epidemiological pattern described for Georgetown, of explosive onset, brief duration, uniform age incidence, high attack rate among users of town water only, bacteriologically normal water samples and absence of known pathogens in the stools.

In February, 1936, there occurred at Coshocton, Ohio, (4) an outbreak similar to those in Georgetown and in Cohasset, no contamination of the system being indicated bacteriologically despite a very definite distribution of cases on the local supply.

The localities so far mentioned have been small cities, towns or villages, all of which used ground water.

One large city, Milwaukee, suffered an outbreak, or a series of outbreaks, which resembled in many respects those outlined above. The most noteworthy one occurred in February, 1938 (5). Neither at this time nor in a previous outbreak, in 1936, were confirmed coliforms found in appreciable numbers, though there were elevated total counts and numbers of gas-formers, together with other evidence that water could not be exonerated.

Outbreaks in Georgetown, Cohasset, Coshocton and Milwaukee occurred with slight, if any, evidence of sewage pollution of the treated water. They had other elements in common, for they occurred in the late fall, winter or early spring, when the weather was cold. Of the four, only Milwaukee used a surface supply, although the Georgetown supply might be considered of surface origin in that the Royal Spring is apparently fed by surface streams which are subterranean for at least a part of their course. Three of these occurrences show an interesting aggregation of cases during or just after week-ends. This fact will be merely noted here and is to be discussed more fully below.

Group II. Two outbreaks--Navarre and the Indiana town reported above--have already been presented as probably due to sewage contamination but without subsequent cases of typhoid fever.

The literature offers a limited number of examples of known, or putative, sewage pollution outbreaks not followed by typhoid fever. In the group to be discussed the evidence rests upon the finding of coliforms in all, or at least in a fair proportion of, samples if not upon the actual demonstration of a relationship between the water supply and sewer system.

Another instance was that of the "Tennessee Town" (6) with periodic outbreaks in June, July and August of 1936. Evidence that sewage contamination of the raw water occurred is presented, though the treated water was felt to have been relatively safe.

In Dallas, (7) Texas, a single water main serving about 10,000 persons became polluted and occasioned a severe outbreak of gastro-enteritis in April, 1937.

In Altamount (8) New York, there occurred a village-wide epidemic of a similar nature in May, 1937.

In Vinton, (9) Iowa, in February, 1938, there was an outbreak of typical gastro-enteritis.

Noteworthy is the fact that only three of the six outbreaks in this group occurred in the winter.

In many of the outbreaks discussed in both groups up to this point reference is made to dysentery-like symptomatology along with the gastro-enteritis, but only in Cohasset, Georgetown, Altamount, and Dallas was there a record of a definite study of stool specimens and in none of these were possible pathogens found. Typhoid fever, noted above as developing not infrequently after this type of outbreak, was not observed in the wake of any of these epidemics.

Group III. There are several instances in which typhoid fever did develop after gastro-enteritis outbreaks traceable to contamination of drinking water. One of the classic examples is that of the "Excursion Boat Epidemic", reported by Lumsden, (10) in July, 1912. The succinct account of the gastro-enteritis phases of this outbreak is well worth reproducing verbatim:

"From Clinton (including Lyons) about 1,200 persons went on the excursion. Of these persons the writer estimates, from the data collected by Dr. Sugg and himself, at least 600 (or 50 per cent) became ill between 12 and 72 hours after their return from the trip. The illness was manifested usually by nausea and vomiting, diarrhea, and prostration. Diarrhea was the most constant symptom. The majority of the cases had nausea and vomiting along with the diarrhea. A few had nausea and vomiting without diarrhea. A small proportion had fever during the diarrheal attack. Some had rather severe abdominal pain. In the majority of cases the duration of the gastro-intestinal disturbance was from 3 to 5 days. In some cases the attack continued for only about 24 hours and in others for several weeks. In some of the cases there were recurrences at intervals of 3 or 4 days. The symptoms presented in the attacks were similar to those which have been presented in a number of outbreaks of diarrhea (sometimes referred to as outbreaks of "winter cholera") resulting from the use of water supplies polluted with sewage. Striking examples were furnished by the outbreak in Mankato, Minnesota, in 1908, (11) and the one in Rockford, Illinois, in 1912 (12).

"Among the residents of Clinton who did not go on the excursion trip on the steamer G. W. Hill on July 29 there was during the summer of 1912 no unusual occurrence of diarrheal disease -------------.

"The writer canvassed in Clinton about 50 households, some of whose members went on the excursion. The outbreak was sharply confined to those who went on the excursion. In a number of instances every member of a family who made the trip was attacked and every member of the family who did not go on the excursion was exempt."

This outbreak was considered to have resulted from pumping river water into the steamer's drinking water tank while the vessel was docked over a sewer outlet. Eleven cases of typhoid fever followed the gastro-enteritis.

In January, 1924, the city of Santa Ana, (13) California, suffered a severe outbreak of gastro-enteritis followed by an epidemic of at least 226 cases of typhoid fever. Cases of paratyphoid, dysentery and paradysentery were also diagnosed.

Detroit, (14) in February, 1926, had a similarly widespread outbreak which was, however, followed by the comparatively small number of eight cases of typhoid fever.

In the summer of 1926 an extensive outbreak of gastro-enteritis, followed by dysentery, paratyphoid and typhoid fever occurred in the urban district of greater Rostov On-the-Don (15) in Russia. There were 2978 cases of recognized typhoid and paratyphoid fever in this outbreak.

The Chicago stock-yard fire on May 19, 1934, gave rise to a most interesting outbreak, reported by Hardy & Spector (16). While fighting the fire a number of firemen drank polluted water and experienced mild attacks beginning one to three days later, characterized by nausea, occasional vomiting and diarrhea. A larger number were reported to have had more intense symptoms lasting for several weeks. Evidence is presented incriminating Endamoeba histolytica in this latter type of case. Sixty-nine cases of typhoid fever and two cases of paratyphoid fever were also reported. A useful description of the gastro-enteric features of the outbreak is included in this report and a new symptom added to the syndrome--progressive loss of weight. This symptom will be mentioned again below.

An outbreak, carefully studied from the laboratory point of view by Ziegler, (17) occurred at Springfield, Missouri, early in July, 1936. Cases were described as having less uniform symptomatology than in the epidemics just outlined. Organisms not fermenting lactose, or fermenting it slowly, were isolated from water samples and from some of the stool specimens.

In December, 1940, an old cross connection was briefly opened in Rochester, (18) N. Y., allowing polluted water from a high-pressure fire control system to flow into the regular water supply mains. The cross connection was open from about 4:30 P.M., December 11th, to 8 A.M. December 12th. More than half the gastro-enteritis cases canvassed had onsets on December 13th and 14th and six cases of typhoid fever followed in due course.

It is notable that in this last group of outbreaks, featuring typhoid fever cases, reports of other enteric syndromes such as dysentery and paratyphoid fever are more prominent. In the first two groups discussed--those with no evidence of sewage pollution and those with evidence thereof, but not followed by typhoid fever--much milder syndromes with no mortality and with a greater predominance of gastric symptoms (nausea and vomiting) are detailed.

As noted above more data are available on these outbreaks in appendix V. Information on dates of the beginning of epidemics has been included there because there seems to be a tendency for outbreaks to start on, or very shortly after, a weekend or holiday. This was the case in Georgetown, Navarre, Cohasset, Coshocton, Dallas, Altamount, Milwaukee and in the "Tennessee town" (where weekend outbreaks were noted periodically during the summer). In Milwaukee daily case totals increased markedly on Mondays for three successive weeks. Two outbreaks starting in midweek--those in the "Indiana town" and in Rochester--followed repair work on the respective systems. The implication of this weekend and holiday grouping is obvious when it is remembered that even in large cities the operating staff of water works is generally reduced at such times and vigilance consequently relaxed.

One outbreak, epidemiologically resembling the milder form described above, has not been included with them because it was attributed to another cause. On May 6, 1939, an epidemic of nausea and vomiting, with or without diarrhea, occurred in one section of Olympia, Washington (19). Although back-siphonage was found to have occurred at the local school, no bacteriological evidence of pollution was found and the outbreak was attributed to volatile poisons from the painting of a water tank restored to service on May 4. School children were affected primarily and the onsets of cases were noted as occurring in the early morning hours--a rather delayed onset for most chemical intoxications. No mention is made of tastes or odors in the water likely to be associated with volatile chemical poisons.

The European literature has been but lightly considered in the above discussion. Papers by Hornung (20) and by Rimpau (21) describe outbreaks. Kathe and Konigshaus (22) and Knorr (23) also deal with the subject. The study of the Hanover Typhoid Outbreak by Mohrman (24) contains some data on gastro-enteritis.

A review of the literature should not be ended without mentioning the stimulating contribution by Schaut (25). This author cites a body of evidence to show that organic cyanides of vegetable origin may be present in water and urges that these be considered as a possible cause for gastro-intestinal outbreaks originating from water. Further observations on this will be made below.

The conclusion derived from field work and from a review of the literature on the subject is that waterborne gastro-enteritis is a definite infectious entity quite distinct from the dysenteries, paratyphoid and typhoid fevers. Because it has an incubation period it appears to be a virus or bacterial infection rather than the result of a chemical intoxication. It should be noted, however, that there is theoretically a hybrid between the infectious and toxic hypotheses which cannot be ruled out on the basis of this reasoning: quite possibly the ingestion of water containing an organism capable of rapidly producing hydrogen sulphide, cyanide, or one of several amines, or other decomposition products in the intestinal tract, could produce a syndrome of this sort.

The disease is not readily communicable from person-to-person by contact. The varied symptomatology suggests a complex of infections rather than a single type, but this variation in symptoms may be the result of variations in dosage, or of idiosyncrasy. The etiological agent, or agents, have not been identified. The findings in Springfield, Missouri, are suggestive but have not been confirmed in other outbreaks.

That the disease is spread by water and often by sewage pollution of water is definitely established. However, there is room for some doubt as to whether sewage contamination is the sole source of the infection, in view of the occasional instances in which no such pollution could be demonstrated. The experience in the field and a review of the literature leave but two deductions on this point: either (a) sewage pollution is a common but not an only source, or (b) the bacteriological standard, with its heavy emphasis on coliform detection, is not an infallible index of water conditions affecting the prevalence of gastro-enteritis. Both points of view will be found expressed in the literature and both may, of course, be correct.

As some of the laboratory studies conducted by the unit bear upon these questions, they will be discussed after these studies have been presented in the next section.

VI. Experimental Laboratory Findings

Because outbreaks were few in number and because several leads were obtained in the course of field work, laboratory studies were carried out. Among the most pertinent of these were observations upon the effect of Berkefeld-filtered sewage taken orally by human volunteers.

1. Experiments with Berkefeld-filtered Sewage.

Fresh human sewage from a sewer pipe draining a populous hillside in Cincinnati was used for these experiments.

The first experiment was carried out with a single volunteer who drank 250 ml. of sewage filtered through a Berkefeld N filter. The filtrate was clear, contained fewer than two coliforms per 100 ml. (no gas formation at 48 hours at 37° C. in five 10 ml. portions planted in lactose broth and the number of bacteria growing on plates after 48 hours incubation was about 5 per ml.); gram-stain of the sediments in the lactose broth tubes revealed only gram-negative elements--chiefly Pseudomonas and vibrioid organisms. However, a small spirochaete was also detected by dark-field examination and found to be the most abundant organism in the filtrate.

Thirty-two hours after taking this filtrate the volunteer developed a profuse watery diarrhea lasting 18 hours, and a few hours after this began he became nauseated and retched without actually vomiting. Temperature was normal or subnormal at the start and rose to 101° F. after a considerable period of diarrhea with dehydration. Extensive examination of four stool specimens upon a wide variety of media revealed no significant organism. A rather surprising finding after recovery was a progressive loss of weight from 180 lbs. to 170 lbs. in the ensuing ten days.

Re-examination of the sewage filtrate after being stored in the ice box at 40° F. for 48 hours revealed a 20-fold increase in the bacterial count which was largely due to proliferation of Pseudomonas. Some strains of this genus are known to be cryophillic.

Three months later another lot of sewage was filtered through two Berkefeld N filters and ingested by four volunteers--one of them the original recipient of the material described above. Filtration was less efficient this time, filtrates from both filters showing an M.F.N. of 13 coliforms per 100 ml. and a count of 160 bacteria per ml. at 48 hours. Color-producing Pseudomonas were detected in one filtrate but none were isolated from the other, though organisms with similar cultural characters without color-production were obtained from the latter. Vibrioid organisms were not found but spirochaetes were abundant in both samples. As in the previous experiment, the only other organisms found in these filtrates were gram-negative.

Two volunteers drank 250 ml. and 50 ml. respectively of one filtrate and two received similar amounts of the second. The original volunteer experienced an attack identical with the one he experienced in the first test, even to the length of the incubation period. His weight loss subsequently was, however, more marked (15 lbs.). Another volunteer was nauseated and vomited twice after a 34 hour incubation period. The other two experienced nausea and diarrhea, with malaise, abdominal cramps and constant, dull abdominal pain for 12-18 hours. One of these last also showed a progressive weight loss during the period following convalescence. Spirochaetes, very similar in appearance to those found in the lactose broth cultures of filtrates, were observed in one freshly-passed stool. No known pathogens were detected upon extensive study of stool specimens from these patients.

A final experiment was performed with six volunteers drinking from 50 ml. to 250 ml. of sewage filtered first through a Berkefeld N and then through a Berkefeld W filter. The filtrate produced no visible growth in lactose broth tubes at 48 hours and was considered virtually sterile. It remained in the icebox during this period and was taken by the volunteers only after this fact had been ascertained. Five volunteers showed no effects, but the sixth, who had received only 50 ml., developed marked nausea and a mild diarrhea without fever after an incubation period of 48 hours. Samples of the filtrate used in this experiment planted in lactose broth developed a faint haziness on the third day, and by the sixth it was evident that all of the six 10 ml. and three out of six 1 ml. portions contained either vibrioid organisms, or spirochaetes, or both. Pseudomonas was not found in this filtrate.

The information furnished by these data on the causative agent of the disease is fragmentary and inconclusive. The spirochaete was the only organism consistently found in all the filtrates, and the possibility that it was responsible was, perhaps, the best lead obtained. Such an organism would not be expected to develop on the agar media ordinarily used for isolation of intestinal pathogens, thus accounting for the failure to find causative agents in stools. The incubation periods observed are, however, very short for a spirochaetal disease.

In this connection it should be noted that Kathe, (26) and Prausnitz & Lubinski, (27) have described an entity, designated "Schlamm fieber," with an incubation period of very short duration to which a spirochaetal etiology is ascribed. Wolter (28) endeavored to attach this diagnosis to the gastro-enteritis preceding the Hanover outbreak.

While these experiments failed to reveal the responsible agent they are of considerable importance for they reproduced in the laboratory, under somewhat controlled conditions, attacks very similar to those witnessed in the field--and the filtrates, it will be noted, showed variations quite comparable to those encountered in the field so far as coliform tests were concerned. They support the field experience that sewage contains an agent giving rise to gastro-enteritis which is not ordinarily isolated on the media commonly used for the study of enteric disease. Unless the 48 hour period of retention of the material of the last experiment in the ice-box destroyed infectious or chemical elements, these experiments also disprove the virus as well as the chemical theory of etiology. Finally, they indicate that the agent responsible for at least one form of gastro-enteritis may be considerably more filterable than the coliforms used as an index of sewage pollution.

More intensive studies of sewage filtrates and of their experimental effects are clearly desirable.

2. Experiments with Pseudomonas. Certain experimental studies were also made upon strains of Pseudomonas encountered in the field and laboratory studies above described. Methods of quick isolation and identification of Pseudomonas strains were evolved. These organisms grow readily on most of the media used for stool examination and water analysis. Streaked on nutrient agar, the green or blue color production was found to be inconstant after incubation at 37° C. but to be quite dependable when incubated at 20°, or when 37° cultures were left for a few hours in the ice box. Upon opening closed petri-dish plates containing cultures of several strains, a distinct odor of cyanide was often observed, and amounts of cyanide up to 0.6 parts per million were removed from broth cultures growing under partially anaerobic conditions by the methods outlined by Patty (29).

It is not impossible that at least some of the cyanide noted by Schaut (25) in waters may be derived in part from this ubiquitous organism.

LaCorte (30) has stated that filtrates of gelatin cultures of *Ps. pyocyanea* produced death with bloody diarrhea when injected introduodenally into animals.

In the laboratory studies which follow, filtrates of lactose broth and of gelatin cultures of *Pseudomonas* strains were taken orally on an empty stomach by three human volunteers. Subsequently whole cultures containing several millions of organisms were also taken in the same way by two of these volunteers. No nausea, vomiting, diarrhea, or cramps were noted in these experiments with whole cultures.

When filtrates of cultures are taken in amounts from 5 to 20 ml. in 50-100 ml. of water, a definite local anaesthesia of the tongue, fauces, and pharynx is noted which persists for several hours. There is a transient feeling of epigastric soreness. For one or two days after the ingestion stool specimens, streaked on MacConkeys agar, may show little or no growth (even when heavy innocula are used).

When whole cultures of *Pseudomonas* are ingested the organism may or may not appear in the subsequent stools. These frequently showed, however, the same relative lack of growth on MacConkey's agar that was noted in experiments with filtrates. When *Pseudomonas* was recovered from the stool it was quite likely to be in pure culture at first, gradually being replaced by coliform colonies in subsequent stools. The incompatibility between *Pseudomonas* and coliforms in common culture is not limited to the human intestine. *Pseudomonas* cultures are known to exert a bacteriostatic, if not actually a lethal, effect, upon many other bacteria in vitro. West (31) has suspected a limitation of the standard bacteriological test for coliforms where *Pseudomonas* organisms occur as contaminants with coliforms in water samples on this basis. The subject merits further study.

3. *Clostridium Welchii Toxin.* Because *Cl. welchii* was once suggested by Nelson (32) as a cause of diarrhea and because of the late gas formation noted in Georgetown water samples, experiments were conducted with four

specimens of Cl. welchii toxin kindly forwarded by the National Institute of Health. Amounts of each up to 40 ml., diluted to 100 ml. with distilled water, were taken by mouth by a single human volunteer without notable gastro-intestinal effects.

4. Algal Toxicity. Blue-green algae have recently been recognized as contributing more than tastes and odors to water. Fitch, et al, (33) have emphasized their importance from the point of view of domestic animals. Strell (34) notes the production of cyanide by blue-green algae as a matter of common knowledge, but unfortunately gives no basis for his belief.

Studies of some of the more common blue-green algae were accordingly begun. None was found to have any toxic effect when given to animals by mouth. One--Microcystis aeruginosa--was repeatedly found to be lethal for mice and guinea pigs when injected parenterally. The toxicity was greatly increased when the algae were frozen, or frozen and dried in vacuo, for preservation. The toxic substance could be dialyzed and withstood autoclaving so that it was considered to be a chemical poison rather than a bacterial toxin. Dilute solutions remained toxic after coagulation with alum, settling, laboratory filtration, and chlorination, but when comparatively large amounts of carbon were used as an adsorbent the solution was detoxified. When the toxic substance was partially purified and administered parenterally to mice the lethal dose was found to be comparatively small - 0.4 mg. Cyanides were at no time detected in algal material.

VII. Summary of Findings on Water-borne Gastro-Enteritis and Discussion.

The studies of the unit have added their quota to the evidence that a form of epidemic gastro-enteritis is actually water-borne; that there is a definite incubation period of 30-48 hours; that the condition is an infection, not an intoxication; and that such outbreaks may occur with little or no evidence of sewage pollution by standard bacteriological tests of water applied within five days of the time such pollution must have occurred. They have shown that the symptomatology and epidemiology of at least one outbreak with no evidence of sewage pollution were indistinguishable from one in which evidence of such pollution was found.

The experiments with Berkefeld-filtered sewage have revealed a syndrome in human volunteers which is indistinguishable from that encountered in the field studies of outbreaks. The fact that a careful search was made for accepted pathogens on an assortment of media, and that none were found, clearly indicates that one or more unrecognized organisms may be responsible.

Less conclusive, but highly suggestive, were two other findings from the review of the literature and of the outbreaks observed by the unit: a tendency for outbreaks, where sewage pollution could not be demonstrated, to occur in the late fall or winter months and a tendency for outbreaks to occur after week-ends or holidays when not directly related to repair work on the water treatment or distribution systems.

Inference, duly labeled as such, is more often required in the interpretation of observations on human experience than of purely laboratory findings, because the observation of human experience can seldom be controlled adequately or completed. When the shortage of supervisory staff in most water works on holidays and week-ends is considered, it is not difficult to supply an inference for the aggregation of outbreaks at such times. The maintenance of a high quality of treatment and of uniform distribution cannot be left to automatic controls or to half-staff supervision.

Several questions are raised by the other observations for which solutions can only be provided in the form of hypotheses. The occurrence of outbreaks without evidence of recent sewage pollution brings out the question contained in the deductions mentioned at the end of Section V: Does the agent of water-borne gastroenteritis exist elsewhere in nature than in human sewage, or is the bacteriological test for coliforms less indicative of pollution that may give rise to gastroenteritis than is generally believed?

In this connection a hypothesis can be formulated to explain the various observations noted above in the field studies of the unit and in the reports of others who have explored the subject. A study of the months of onset of outbreaks shows that where the outbreaks resulted from polluted water they occurred either in winter or in summer. The limited number of coliform-negative outbreaks all occurred in cold weather. The numbers are small in both instances and definite conclusions cannot be drawn. However, there seems a possibility that in cold weather coliforms occasionally may be replaced rather rapidly after they gain admission to water mains by hardier forms which, alone, are found by the time samples are collected and analyzed. With a one to three-day incubation period before gastro-enteritis supervenes and a one to three-day period before investigation is begun, this hypothesis could explain the occasional absence of coliforms in collected samples, if it were known that other organisms found in sewage can displace or overgrow coliforms under conditions of "cold storage". Reports on this subject are rather few but some are suggestive. Parr (35) cites a citrate-utilizing slow lactose fermenting coliform capable of rapidly displacing other types of coliforms in feces stored in the ice box. That certain strains of Pseudomonas do grow readily at low temperatures in well known. The cultures and filtrates of cultures of some of these latter organisms are known to have a bacteriostatic, if not an actual lethal, effect on other microorganisms, and indications are not lacking (31) that the presence of this organism may prevent the identification of coliforms in the standard presumptive coliform tests. Studies at present under way (36) indicate that a considerable preponderance of Pseudomonas is needed to achieve complete suppresion of gas formation, and it may be doubtful whether such a preponderance could have been achieved in the water mains during the short period before the Cohasset, Georgetown, and Coshocton outbreaks came under study.

The above hypothesis is a rather tenuous one but offers some grounds for detailed studies in view of the implication it carries as to the fallibility of the standard bacteriological test to demonstrate pollution in the recent past under cold weather conditions.

According to this view the standard coliform test, if applied at the time of pollution, would still suffice, but cannot be relied upon indefinitely, particularly in the presence of certain other organisms.

What supplementary tests might be employed in the occasional instance where pollution cannot be demonstrated by standard methods? The laboratory studies suggest two organisms that might be regarded with suspicion until further work exonerates them or implicates one, or both, more definitely -- the Pseudomonas and the spirochaete. Both are apparently present in sewage, both survive fairly drastic filtration.

The above supplementation is not necessary for routine analyses of water quality and is suggested simply as an adjunct of epidemiological study in the rare instances where routine methods fail. Such failure would appear to be due to delayed application rather than to inadequacy.

The implication from the weekend onsets of outbreaks is that present filtration and chlorination standards are adequate for the control of water-borne gastro-enteritis from polluted sources if these standards are consistently met.

VIII. Studies of Diarrhea and Enteritis

As stated in the first section of this report, a further aim of the unit was to determine to what extent the source and quality of the drinking water of communities might contribute to the prevalence of "diarrhea and enteritis", commonly confused with the syndrome described at length in the preceding sections.

For this purpose 144 cities of 10,000 or more population throughout the Ohio River basin were selected. As complete morbidity data for diarrhea and enteritis are non-existent, mortality data were selected to serve the ends of the study. The annual "Mortality Statistics" (37) volumes served as the source of these data. For each of the cities, deaths under the following rubric were taken off for the years 1900-1937. (*) "All causes", "Diarrhea and enteritis" (for all ages, and specifically under 2 years) and "Typhoid fever". In order to obtain annual rates of mortality, population figures for each city were obtained from the decennial census volumes, simple linear interpolation being used to obtain population in intercensal years. Because there were wide fluctuations, particularly in the "D. & E." rates for a given locality from year to year, five-year median rates were derived for certain purposes.

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* Certain cities attaining their 10,000 "majority" after 1900 were included where data over a sufficient interval could be obtained.

Where rates are used as indices of variation, it is customary to apply corrections in order to eliminate effects incidental to the chief cause of variation. Differences with respect to age, sex and color were studied in sample cities and found to involve minor corrections, if any. On the other hand relatively important corrections were indicated in many cities on the basis of residence. Many of the larger cities tended to accumulate patients from surrounding rural areas or near-by smaller cities, (hereafter called "satellite cities") with the result that rates for the former were unduly high. In the satellite cities of the study, on the other hand, rates were often unduly low--sometimes actually zero for quite long periods. The residence death correction could not be made specifically for diarrhea and enteritis deaths -- no such data being available -- but was based upon deaths from all causes from 1937 and 1938 "Mortality Statistics" data on resident and non-resident deaths from all causes in each city.

It is recognized that the rubric "diarrhea and enteritis" is so comprehensive that a subclassification would be highly desirable. It probably includes a great many conditions quite incidental to infectious, or even to non-infectious, conditions of the intestinal tract and it does not include all dysentery deaths, which would be highly pertinent to the investigation at hand. However, deaths specifically classed as dysentery were so few that their omission from the rates was found to make unimportant changes, and this item was accordingly omitted. Actually most of the severe or fatal cases classed as "D & E" have been found to be undiagnosed dysentery. One of the important causes of diarrheal death in the South is probably undiagnosed pellagra -- a point which should be carried in mind when the relatively high rates in this region are being considered. However, dysentery deaths are also more frequent in the South than in the North and undoubtedly still constitute the bulk of the deaths from D & E.

The method of the study consisted in comparing the rates for groups of cities, each group differing with reference to some important aspect of water source or treatment. Comparison was also made between typhoid fever and diarrhea and enteritis rates in order to determine whether the installation of various improvements in water treatment might have affected one more than the other. Finally certain detailed studies were made of water quality in relation to diarrhea and enteritis mortality in individual communities.

In general, there are two sources of urban water supply; well water, hereafter called ground supply, and surface water from streams, creeks and rivers. Many cities, however, have both, using one or the other as supplementary source or continuously use the two in combination. Enough cities used these combined supplies to warrant a third category hereafter referred to as "mixed supply".

Table 1.

Averages and medians of the 1933-1937 mean diarrhea and enteritis death rates per 100,000, of cities in the Ohio River Basin as a whole and by regions, according to source of water supply.

Area	Surface	Mixed	Ground
A - Averages of Mean Rates			
Total O.R.Basin:	21.6	14.3	19.0
Northeast	12.3	11.1	11.3
Northwest	26.4	19.8	22.5
South	39.0	28.4	33.5
B.- Medians of Mean Rates			
Total O.R.Basin	13.7	15.4	16.0
Northeast	11.6	10.4	11.2
Northwest	20.3	18.7	21.3
South	33.0	28.4	35.8

For a comparision of rates in cities using these three categories of water, the annual rate for diarrhea and enteritis for each city over the period 1933-1937 was derived, and the mean rates for all the cities in each group were averaged to obtain a mean rate for the group. The comparison is shown for cities in the Ohio River Basin in the first line of Table 1.

The variation between rates in individual cities of the Basin is very marked. It is often quite extreme when the rates for adjacent cities are compared. This subject will be discussed at greater length below. However, in general the rates tend to be lowest in the northeastern section (southern New York, western Pennsylvania, eastern Ohio). They become higher in the northwestern section (western Ohio, Indiana and eastern Illinois) and tend to be very high in the southern section (West Virginia, Kentucky, Tennessee, northern Alabama). Ground water supplies are more frequently used in the two northern sections and surface water supplies more commonly in the South. There is, however, a fair number of both in each of the regions so that a regional comparison would appear to be indicated. This has been made in the next three lines of Table 1. Here, only a slight excess mortality appears in the cities using surface supplies when compared with those using mixed or ground supplies. The variation by region is seen to be quite striking -- averages for the Northwest and for the South being about double and triple, respectively, those for the northeast in each category. Mixed supplies tend to show lower rates than either ground or surface, but there are relatively few of these.

Because even the mean rates for cities over a five-year period show rather wide variation, the median offers perhaps a better basis for comparing these rates than the average. The second part of Table 1. shows median data for the same groups of cities. Here very little difference appears between the various sources of supply; the basic difference is again unquestionably the regional one.

Ground water supplies in general are considered less subject to pollution than surface supplies. Like all generalizations, this has its exceptions, but it holds true.

A further study, supplementary to the above, was made of the variation in mortality from diarrhea and enteritis with variation in the quality of raw water for cities using surface supplies. The D & E deaths in individual cities were compared with the monthly average figures for the M. P. N. of coliforms, and plate counts, in the raw water of individual cities using surface water as a source of supply over a considerable period of years. Such data were available for Cincinnati, Ohio, and for Louisville, Kentucky. In general the coliform M. P. N. and plate counts tended to run low in the summer and higher in the winter in the raw water of both of these cities, while the D & E deaths were concentrated in the summer months and declined notably with the onset of cold weather. On a monthly basis, therefore, no definite relationship could be found between the quality of the raw water as indicated by the M. P. N. of coliforms and the plate counts.

A final comparison--that based on the quality of treated water--would be perhaps more pertinent to the present study. However, very nearly all of the 144 cities consistently met the U. S. Public Health Service standard over the period 1933-1937 and little basis therefore exists for the establishment of subdivisions based on treated water quality. A majority of cities also used equipment and techniques of water treatment of a sufficiently high order so that grading them upon such a basis would entail division into groups differing in such minor respects as to be unimportant.

One of the early proofs that typhoid fever in cities was traceable to polluted water supplies, was obtained by the demonstration of consistently, and often strikingly, low rates following the installation of water treatment procedures, such as filtration and chlorination. Typhoid fever death rates prior to and subsequent to the water treatment installation formerly were compared and decreases in the latter as compared with the former were noted with enthusiasm. The same favorable effect was often discernable in the death rate for all causes (Mills-Reinecke phenomenon). The effect on the typhoid fever rates was often definite and unmistakable. The effect on the general death rate was less clear-cut, and it was soon recognized that, where a rate was consistently declining--

as the general death rate from all causes was declining in most cities during the latter part of the nineteenth and in the early part of the twentieth centuries -- some decrease inevitably occurred when the rate for a later period was compared with that for an earlier period.

The effect of the installation of improved water treatment methods on the typhoid fever death rate seemed definite enough, however, to provide a guage for the extent in which diarrhea and enteritis deaths may have been, to former year, ascribable to water. With this in view, three groups of cities using surface water supplies and beginning water treatment in the interval 1905 to 1933 were studied in detail. The first group consisted of seven cities (Table 2) instituting filtration and chlorination in combination. The second contained 14 cities (Table 3) where filtration was started independently of, and usually prior to, chlorination. The third contained 22 cities (Table 4) where chlorination was begun independently of, and usually subsequent to, filtration. Groups 2 and 3 contain many cities in common. diarrhea and enteritis mortality rates were computed for individual years for the five years preceding and the five years subsequent to the installation of treatment (omitting the year in which treatment was begun). Because the D & E rates show a consistent downward trend over the whole interval 1900-1937 it was to be assumed that the subsequent interval would show lower rates whether water treatment was related to D & E mortality or not. As a control, similar rates for typhoid fever mortality were computed for comparison with those of D & E.

Averages of the five-year annual rates for the periods before and those after installation were at first used, but variability in these intervals prompted the use of the median rather than the mean. Both methods were in essential agreement, so the median rates were selected for presentation. The resulting tables show the median D & E and typhoid fever mortality rates for the two intervals before and after beginning treatment as follows: Table 2, cities beginning filtration and chlorination in combination; Table 3, cities instituting filtration separately; Table 4 cities instituting chlorination separately.

It will be observed that the five-year median rates for cities in the first group (Table 2) uniformly

Table 2

Median mortality rates per 100,000 for Typhoid Fever and for Diarrhea and Enteritis for the five-year periods before and after combined Filtration and Chlorination in seven cities using surface supplies.

	D & E Rates			Typhoid Fever Rates		
City	Median 1-5 years before	1-5 years after	Ratio After/Before	Median 1-5 years before	1-5 years after	Ratio After/Before
Logansport, Ind.	70.3	34.4	0.49	37.9	34.0	0.90
Cambridge, O.	50.0	39.8	0.80	40.3	7.8	0.19
E. Liverpool, O.	104.2	61.0	0.59	76.6	32.8	0.43
Ironton, O.	93.9	45.5	0.48	65.9	13.0	0.20
Portsmouth, O.	94.1	90.5	0.96	70.9	28.3	0.40
Steubenville, O.	156.3	143.0	0.92	67.8	22.0	0.32
Wilkinsburg, Pa.	93.3	35.0	0.37	47.6	18.0	0.38

Table 3

Median Mortality Rates per 100,000 for Typhoid Fever and for Diarrhea and Enteritis for the five-year periods before and after filtration in 14 cities using surface supplies.

	D & E Rates			Typhoid Fever Rates		
City	Median 1-5 years before	1-5 years after	Ratio After/Before	Median 1-5 years before	1-5 years after	Ratio After/Before
Anderson, Ind.	91.1	68.9	0.76	72.7	28.1	0.39
Evansville, Ind.	87.3	75.1	0.86	34.1	23.6	0.69
Indianapolis, Ind.	123.2	73.4	0.59	43.5	31.3	0.72
New Albany, Ind.	60.9	53.9	0.89	23.4	18.1	0.77
Louisville, Ky.	72.8	52.0	0.72	63.6	26.7	0.42
Paducah, Ky.	203.2	81.5	0.40	85.2	65.3	0.77
Bellaire, O.	110.5	48.5	0.44	36.2	20.5	0.57
Cincinnati, O.	109.2	99.9	0.91	61.2	13.3	0.22
Columbus, O.	60.1	55.2	0.92	35.8	18.8	0.52
Marietta, O.	52.8	53.6	1.02	98.0	23.1	0.24
Youngstown, O.	115.9	169.9	1.47	123.7	36.3	0.29
McKeesport, Pa.	196.8	159.4	0.81	107.1	32.5	0.30
Wilkinsburg, Pa.	93.3	46.2	0.50	47.6	34.0	0.72
Nashville, Tenn.	55.1	35.8	0.65	16.1	7.7	0.48

Table 4

Median Mortality Rates per 100,000 for Typhoid Fever and for Diarrhea and Enteritis for the five-year periods before and after Chlorination in 22 cities using surface supplies.

City	Median 1-5 years before	D & E Rates 1-5 years after	Ratio After/Before	Median 1-5 years before	Typhoid Fever Rates 1-5 years after	Ratio After/Before
Evansville, Ind.	75.1	32.2	0.43	23.6	4.5	0.19
Indianapolis, Ind.	73.4	68.0	0.93	31.3	24.0	0.77
New Albany, Ind.	53.9	38.7	0.72	22.2	4.3	0.19
Terre Haute, Ind.	111.8	83.2	0.74	37.1	24.4	0.66
Vincennes, Ind.	75.1	54.0	0.72	25.4	24.4	0.96
Louisville, Ky.	52.0	50.2	0.97	24.0	14.4	0.60
Paducah, Ky.	81.5	76.4	0.94	60.5	24.8	0.41
Asheville, N. C.	58.7	48.3	0.82	18.3	7.6	0.42
Bellaire, O.	116.1	47.6	0.41	20.5	7.0	0.34
Cincinnati, O.	110.3	77.1	0.70	13.3	6.6	0.50
Columbus, O.	47.6	38.0	0.80	13.9	4.5	0.32
Marietta, O.	28.5	33.5	1.17	29.9	20.4	0.68
Newark, O.	42.9	44.4	1.04	35.1	7.4	0.21
Springfield, O.	41.6	30.3	0.73	26.2	6.4	0.24
Warren, O.	49.0	73.9	1.51	28.6	33.3	1.16
Youngstown, O.	14.2	8.3	0.58	1.2	1.2	1.00
Beaver Falls, Pa.	133.6	103.5	0.78	16.7	48.0	2.87
Du Bois, Pa.	80.4	40.2	0.50	14.7	7.6	0.52
McKeesport, Pa.	159.4	82.7	0.52	32.2	10.8	0.34
New Castle, Pa.	124.0	83.7	0.68	48.4	21.7	0.45
Uniontown, Pa.	79.6	81.3	1.02	97.7	59.1	0.61
Wilkinsburg, Pa.	68.7	35.2	0.51	45.7	18.0	0.39

fell for the interval after the treatment as compared with those for the period before treatment. However, the decline was not as marked (as shown by higher ratios) as in the case of typhoid fever.

The same is true of Tables 3 and 4 although here, occasionally, higher median rates for both D & E and typhoid fever are evident in the post-treatment interval.

Mean ratios for D & E in the three groups are 0.66, 0.72 and 0.78 respectively. This means that the median rates subsequent to treatment were between two-thirds and three-quarters of the median rates prior to treatment. Typhoid fever on the other hand shows more consistent decreases. Here mean ratios for the three groups were, respectively, 0.40, 0.51 and 0.63. A review of the ratios, city by city, will show that marked decreases in the D & E rates (as shown by low ratios) are not necessarily accompanied by similar decreases in typhoid fever and vice-versa.

The tentative conclusion is that improvement in the safety of drinking water by water treatment in cities of the Ohio River watershed did not affect the D & E mortality to the extent that these improvements affected the typhoid fever mortality. A detailed study of trends would be needed to establish this point more definitely. The era in which these improvements were begun (largely between 1905 and 1925) was one of great activity in environmental sanitation generally. Water treatment was but one of these activities; simultaneously privies were abolished with the extension of sewage systems, garbage was collected and burned or buried, livery stables disappeared, and foods were safeguarded.

Limited as the foregoing observations have been to D & E mortality, they do not rule out the possibility of a considerable morbidity with low mortality in cities with water as the vector. They simply indicate that the mortality listed under the rubric "Diarrhea and Enteritis" is but little influenced in recent years by the source of the water supply when adequate treatment is applied.

These essentially negative mortality findings would be more conclusive if real relationships with other sources or vectors could be demonstrated. Other relationships were sought and suggestive leads were found, though no definite conclusions were drawn.

Forming, as dysentery does, a notable proportion of the severe deaths attributed to D & E and being, as dysentery is, a disease of poverty, it might be expected that economic factors are of importance in the distribution of D & E deaths. Such a hypothesis might well be evolved to explain the prevalence of this group of diseases in the South, and in the crowded sections of the larger cities. However, no clear-cut relationships between D & E death rates, city by city, and various economic indices (income tax payments, magazine sales, etc.) could be found.

Very definite was the relationship between latitude and D & E mortality for cities in the Ohio River Basin. A subsidiary relationship here was found to be altitude. With few exceptions cities located at higher altitudes enjoyed more favorable rates than those at lower altitudes but in the same latitude. This factor was found to explain some, but not all, of the variation between neighboring cities.

These findings suggested that climatic factors may be of importance. A very considerable and conflicting literature on the relationship of diarrhea and enteritis mortality and climate exists, which will not be possible to review here. The studies of the unit on climate in relation to diarrhea and enteritis covered a wide variety of localities. It was noted that D & E deaths when studied for individual states, by months, tended to show fairly narrow, elevated peaks in the late summer (August and September) in the northern states, while the peaks came earlier (May-June) in the southeastern states and tended to have a broader base. In the states of the continental U. S. precipitation is fairly constant throughout the year but temperature varies markedly. The reverse is true in Porto Rico and Hawaii where temperature remains throughout the year at levels reached only in summer in the United States but where rainfalls vary considerably. In Hawaii the rains, according to rather fragmentary data for Honolulu, occur in what would be the winter months. In Porto Rico there are two periods of rain, one in summer and one in winter. D & E mortality is surprisingly high throughout the year in both these localities but small peaks of mortality frequently occur after the rains--which are seldom excessive. The relationship is not a direct one because the mortality maxima tend to follow the peaks of rainfall after an interval of a month or more and do not coincide with them.

When the climatic and monthly mortality record of individual cities of the Ohio River Basin is studied over a long time interval, it is apparent also that hot and rainy summers show a greater D & E mortality than hot and dry (or cool) summers. There appears, then, to be some relationship between this disease and both temperature and precipitation. The lag in the relationship to both temperature and precipitation is, however, noteworthy, and may help to explain some of the negative reports on these factors in the literature where an attempt was made to relate deaths and simultaneous climatic conditions.

Further evidence that the climatic factors are indirectly related is obtained from a comparison of rates for cities not widely separated geographically, yet similar from the point of view of altitude and non-resident deaths. Some will consistently run higher than others. Upon investigation the former will often be found to have water supplies comparable with those of the latter but other sanitary provisions, notably, (sewage and waste disposal) will be found less adequate.

The delayed relationship with climatic factors and the observations just noted give some encouragement for the hypothesis that the housefly--fallen into discredit of recent years as a disease carrier--may actually play something of a role as a vector for some of the diseases included in the broad category of diarrhea and enteritis. Undoubtedly the remainder of the famous complex of food, fingers, and flies, plays some role, aided and abetted by improper care of food (inadequate screening and refrigeration) both at the source of supply, in urban distribution centers, and in the home.

Field observations were made upon diarrheal outbreaks on more than one occasion. They appeared throughout the summer primarily in the form of individual household outbreaks, without the close relation between onsets in other attacked households noted in the explosive water-borne gastro-enteritis epidemics. Children were attacked more frequently and severely than adults, and the mortality tended to be far higher than with gastro-enteritis.

A somewhat puzzling fact about diarrhea and enteritis is that the mortality is considerably higher in male than in female children -- a fact commented upon by Bakwin (38), Hosoi & Alvarez (39) and by Ciocco (40).

In this male preponderance, as in the age and seasonal distribution of cases, there is a close analogy with infantile paralysis. This analogy is even beginning to have regional aspects within the continental United States, at least.

An opportunity for the intensive field study of one diarrhea and enteritis outbreak was presented when the unit was asked to collaborate on the field and laboratory study of an outbreak with a relatively high mortality (117 cases and 12 deaths) in Adair County, Kentucky. With examinations conducted upon fresh stools, a surprisingly high proportion proved to have bacillary dysentery of the Shiga type. The outbreak was at first confined to homes on the outskirts of Columbia, the County seat, beyond the limits of the town water supply where privies (seldom used by the younger children) and poor sanitary conditions of the homes themselves were noted. Well water supplying these homes was found to be polluted, but there was little relation between the onset of cases in homes using water from the same well, and many homes escaped entirely. A survey of non-attacked homes in this area revealed better screening against flies, more meticulous housekeeping and better protection of the food between meals. As the epidemic progressed occasional cases were noted in the better type of home but only three cases occurred in homes supplied with running water from the town system. Cases also began to occur on more or less distant farms -- often after family visits to or from homes in the stricken area -- and outlying foci were set up from which the disease spread to adjacent farms.

Cases occurred at all ages, but children tended to be attacked more often than adults, and the fatal cases were all under eight years of age. Males were more often attacked than females. The mortality was also greater among males, but the case fatality was the same for both: a point of some interest in connection with the sex differences in mortality noted above.

The outbreak of Shiga dysentery in Adair County started in the latter part of May and lasted throughout the summer -- a prolonged affair in comparison with the epidemics described earlier in this section of the report. (See Appendix IV)

While the role of flies in the dissemination of this disease may have been a minor one, it is evidence that the epidemiological picture is hardly that of a water-borne epidemic.

This and other studies made above tend to sustain the view that the role of water as a factor in the mortality complex "Diarrhea and Enteritis" is a negligible one where present standards of water quality are met and maintained continuously.

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APPENDIX I.

Form used in Field Study

45-8

WATER-BORNE DISEASE STUDY

ADDRESS______________________; White___ Colored___; Church______________

Check person (s) interviewed.		Name	Sex	Age	Date First attk'd	Hour First attk'd	SYMPTOMS					Place of occupation or school	Visits away from home during period of outbrk	Water used for drinking
							Naus	Vom	Cramp	Diarrh	Other			
	1		M F											
	2		M F											
	3		M F											
	4		M F											
	5		M F											
	6		M F											
	7		M F											
	8		M F											
	9		M F											
	10		M F											

Guests or visitors present during period of outbreak but now left: ______________ Address__________

Date arrived __________ Date left __________

Household
Water Supply: City___ Well___ Cistern___ Spring___ Other___
Comments on quality of water during period preceding outbreak:
Taste________ Odor________ Color________ Turbidity________ Other______

Milk Supply: Source______________ Comments on quality of milk______________

Food, Baked goods, eclairs, door to door delivery:

Space for special questions and for extension of remarks above:

Date Interviewed__________ Interviewer______________ Cooperativeness__________ (Over)

APPENDIX II.

Gastro-enteritis Outbreak in Georgetown, Kentucky

Epidemiological Study of Water-borne Gastro-enteritis in Georgetown, Kentucky

by

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On the 16th, 17th and 18th of November, 1940, a strikingly high prevalence of gastro-enteritis in the city of Georgetown, Kentucky came to the attention of the Scott County Health Department. Preliminary studies made by that Department indicated that the water supply might have been the source of the trouble. The superintendent of the water treatment plant was asked to increase the amount of chlorine in the city water, and this increase was started at noon on the 18th. The State Department of Health was also notified, and, through the latter's cooperation, the outbreak came to the attention of the Water-borne Disease Study Unit of the Ohio River Pollution Survey on the afternoon of Monday, November 18th. An intensive study of the outbreak was begun on the morning of Tuesday, November 19th.

The study was divided into three broad types of inquiry; (a) a house-to-house canvass of a sample of the population to determine the incidence and distribution of the disturbance and its vehicle; (b) laboratory studies of the suspected vehicle and of patients to determine, if possible, the primary cause; (c) environmental studies of a sanitary character. The present report will deal primarily with the results of the first two of these, the last having been covered in an early separate report owing to the urgency of the need for correcting some of the conditions disclosed.

Results of the House-to-house Inquiry

When questioned as to the character of the illness it was usually described as an acute diarrhea with severe "cramps" and often accompanied by either nausea, vomiting, or both nausea and vomiting. During the canvass 131 cases were discovered, the detailed symptomatology of which is given in Table 1.

Table 1.

Symptons Described for 131 Cases of Gastro-enteritis,
Canvassed Populations, Georgetown, Ky.

Sympton	No. of Cases
Diarrhea Alone	26
Diarrhea and Cramps	61
Diarrhea, Cramps, Nausea or Vomiting	29
Cramps Alone	15
Total	131

In general the duration was given as "a day or two" but a few cases lasted three or four days. In about 10 percent of the cases it was noted that the initial attack subsided in one or two days but relapse occurred with quite violent watery diarrhea lasting another two or three days as though the first attack had paved the way for secondary invaders.

The dates of onset obtained by canvass and by questionnaires addressed to 136 college students are shown in Table 2.

Table 2.

Dates of Onset of Cases of Gastro-enteritis in the
Canvassed Population of Georgetown, Ky., and among
Students of Georgetown College,
November, 1940.

Group	Dates of Onset										
	Prior to Nov. 14	14	15	16	17	18	19	20	21	22	Unknown	Total
College Students	6	3	25	31	26	11	6	-	-	-	3	111
Georgetown Proper	4	3	5	25	30	29	14	9	2	4	6	131
Total	10	6	30	56	56	40	20	9	2	4	9	242

A limited number of cases occurred in Georgetown College after the 19th - the day on which the questionnaire was submitted. Canvassing continued in the city until the end of the week, by which time it was possible to obtain accurate dates of onset for only a few recent cases.

Cases were not uniformly distributed throughout the city, but tended to be concentrated in the better residential districts and in the college. One section of small dilapidated homes inhabited by colored day-laborers escaped entirely, although almost surrounded by a better class of residential homes which were heavily attacked. This poorer section had practically no running water in the home, being supplied almost entirely by small hydrants on the city water supply located near street intersections. Canvassing in this section was quite unsuccessful during daylight hours because few people were at home. The freedom from attack was not wholly racial because colored families up-town where the prevalence was high showed practically the same incidence as the neighboring white families.

The age incidence of the malady was nearly uniform for the various decades of life and is shown in Table 3. There is seen to be no concentration of cases in the younger ages so characteristic of milk-borne outbreaks and of the summer diarrheas. Also against the hypothesis that milk served as the vehicle was the fact that the incidence among those taking from all six of the city's principal milk sources, including one distributing adequately pasteurized milk, was very nearly uniform.

Table 3.

Age-Incidence of Gastro-enteritis Among the Canvassed Population of Georgetown, Kentucky.

Age (Years)	Total Canvassed	Total Attacked	Percent Attacked
Under 2	9	1	11.1
2 - 9	53	10	18.9
10 - 19	101	19	18.8
20 - 29	70	29	41.4
30 - 39	49	13	26.5
40 - 49	57	19	33.3
50 - 59	52	15	28.8
60 - 69	42	19	45.2
70 Plus	25	5	20.0
Not specified	6	1	16.7
All ages	464	131	28.2

Food was bought at a diversity of stores by the various households. There was only one door-to-door food vending agency - a bakery wagon from an adjacent city. The largest part of the sales for this wagon consisted of bread and rolls, and many attacked households reported not buying from this agency at all.

The true incidence of the malady in the population of Georgetown was probably considerably above the figure of 28.2 percent shown for all ages in Table 3 for reasons which will presently be given. So high an incidence could only be explained on one of two hypotheses; first that the vehicle was the town water supply, or second, that an exceedingly infectious virus was spread from person to person as influenza is now considered to be spread.

That the first of these seems more likely is suggested by the incidence among those habitually drinking city water compared with the incidence where it was not habitually taken. Nearly all of those using wells or cisterns for their drinking water supply in Georgetown were interrogated in the house-to-house canvass.

Table 4.

Incidence Among Canvassed Persons in Georgetown Using City Water and Among Those Habitually Using Private Well and Cistern Water.

Source of drinking water	Canvassed	Attacked	Percent Attacked
City Water	388	119	30.6
Other Sources	76	12	15.8

The significance of the difference between the two percentages in Table 4 is considerably enhanced by the fact that for eleven of the 12 persons who were attacked, despite the habitual use of other sources of drinking water, it was possible to obtain a history of fairly liberal use of city water while working or visiting outside the home. Had the disease been an air-borne virus there should have been no such marked discrepancy as that shown in Table 4 between two groups of Georgetown residents.

In addition to including a nearly complete tally of persons using other sources of water supply, Table 4 contains a disproportionately large number in the colored section above mentioned where the incidence was found to be very low. The residential section about Georgetown College and the college itself were the sections where the disorder was most prevalent. Questionnaires filled out by students at the college showed 109 or 80 percent attacked. This whole section of the city was covered by canvass much less completely than other areas. For these reasons the incidence for the city as a whole must be regarded as far higher than the figures shown in Tables 3 and 4. A reasonable estimate would be a figure not far from 50 percent.

During the course of the canvass a number of statements were obtained about the simultaneous prevalence of the disorder among persons on outlying farms, in adjacent cities and even in remote centers. These were investigated, so far as time permitted, and usually turned out to be unfounded or based upon the fact that an illness had occurred which was not the same as that experienced by the residents of Georgetown. Occasionally the true version offered a startling confirmation of the waterborne hypothesis. Not a few cases closely resembling those in the city were encountered on outlying farms as sole cases in large households. Upon questioning it was usually found that the patient had visited Georgetown a few days prior to the attack and some of them could recall having ingested nothing but water while in the city. Where the date of visit and date of onset seemed to be remembered accurately these cases were used to establish incubation periods for the disorder. One case had an onset within twelve hours after a fifteen minute stopover in Georgetown during which he had taken a glass of water and a ham sandwich. A patient in the Georgetown Hospital was discharged to his farm home on the 17th and developed a typical attack on the 20th. Another patient who visited Georgetown briefly on the 16th was attacked on the 18th. Forty-two students from an Indiana college attended a football game with Georgetown College on the 16th at Georgetown. The day was cold and many, even of the players, denied having taken any water during their six hour stay in Georgetown. However, sixteen recalled having taken varying amounts of water; only three drank freely, the others having had one glass or less. Four cases of gastro-enteritis occurred and all four occurred among the sixteen students with a history of having taken water. All but one had also eaten ice cream or "hot dogs". The other recalled having had nothing but water during his stay. The onsets were on the 17th, 18th, 20th and 21st. The player whose onset occurred on the 17th broke training after the game and may have had other causes for his attack of vomiting and diarrhea.

A number of people were questioned as to whether they had noted any abnormal tastes or odors in the water during the week prior to the outbreak, and several answered that the water was unusually malodorous toward the end of the week. The odor was noted particularly on opening bottles of water kept for some time in the refrigerator. Few could describe the quality of the objectionable smell or taste but one intelligent response was that it smelled and tasted "like rotten wood".

Summing up the results of the house-to-house study, then, the evidence strongly indicates that water was the vehicle by which this infection or intoxication was distributed in Georgetown. That it was more probably an infection than an intoxication is evidenced by the two to three day incubation period of the few cases having a short enough exposure in the city to justify drawing a conclusion. The agent was probably in the water as recently as Saturday afternoon, November 16.

Laboratory findings.

During the latter part of the week of November 11th a peculiar blue-black tint was noted in the water of the Royal Spring from which Georgetown receives its water supply. In the settling basins this color was particularly noticeable where the water was not completely obscured by floating masses of decomposing algae. It may be that the latter contributed some of this color for it was noted that the water in bottles in which some of these algae were collected was tinted quite deep blue after standing. The plant masses were found to be largely composed of a species of Oscillatoria. The odor of these samples was very unpleasant and the taste much like that of "rotten wood". Some of this material was autoclaved and ingested with no ill effects. It cannot be said that the algae were entirely incidental to the outbreak, however, for it seems likely that in decomposing they at least added to the organic load of the water. This would help to explain one of the first of the laboratory findings which was that, although the chlorine feed had been markedly increased at noon on Monday, November 18th, no residual chlorine was detected in any of the samples taken at noon the following day with but a few hours detention of treated water at the plant.

Before detailing the bacteriological analyses of the water at the time of the outbreak it may be useful to present the results of the routine analyses of treated city water as shown by the State Health Department Laboratory reports on file in Georgetown for the entire year of 1940. These are detailed in Table 5.

Table 5.

Total Bacterial Counts (37°) and Percentage of Gas Formed in Lactose Broth at 48 Hours in Routine Analyses of Georgetown Water Samples for the Year 1940

Date		Total Count, per cc.	Percent Gas Formed
January	10	5	0
	17	0	0
	24	9	0
	31	9	80
February	7	21	60
	9	108	10
	14	3	0
	21	1,144	0
	28	954	0
March	6	572	0
	10	8	0
	13	508	0
	20	8	0
	27	2	0
April	3	3	0
	10	5	100
	17	1,144	(a)
	24	0	0
May	1	Over 10,000	20
	8	0	0
	21	(b)	(b)
July	10	5	0
	16	572	0
August	1	5	0
	14	2,163	0
	21	954	0
	28	9,158	0
September	4	4	0
	11	(c)	(a)
	17	2	0
	25	2	0
October	2	Over 10,000	0
November	8	5	0
	18	28	90
	20	4	0
	25	2	0
December	2	84	0
	4	0	0
	16	3	0

(a) No record on gas formed.

(b) Four samples from various points showing total counts of 0 or 2 and no gas.

(c) Colored school - 1271; Garth school - 954

There is evidently a very substantial variation in the total count and at times extensive gas formation is noted in the presumptive broth tubes after 48 hours, though the degree to which this latter would confirm is not given. Unfortunately, no sample was sent to the laboratory during the week preceding the outbreak (November 10 to 16). The samples were taken at a tap at the water treatment plant. Some of the variation in total count may be explained on the basis of faulty operation of the filtration plant. However, a decided variation in total count and marked turbidity in the raw spring water is known to occur a few days after heavy rainfall over the collection area feeding the spring. A light drizzling rain had fallen in Georgetown on Armistice Day, five days before the outbreak. There was reason to believe that the rainfall had been heavier south of the town in the region from which the spring receives its water, as the flow was augmented markedly and the water became turbid just prior to the onset of the epidemic. During the ten days following the rain the temperature fell below the freezing point several times.

The first samples available for bacteriological study after the outbreak began were taken by the County Health Department at noon on November 18th, kept overnight on ice and analyzed the following day. The results of examining these samples - taken at scattered points on the main grid of the distribution system are shown in Table 6a. The results of a second set, taken in all but one instance from similarly scattered but slightly different points on the main grid on the following day, are given in Table 6b. The results of a third set taken chiefly from dead-ends on the same day as those in Table 6b (November 19) but studied in a somewhat different manner are given in Table 6c. On the 19th after taking these samples (which showed no residual chlorine despite the increased feeding of chlorine the previous day) the dose was again increased and a residual began to appear in the mains toward evening. Samples taken subsequently showed a heavy residual and were practically sterile.

Except for evidences of high content of gas-forming bacteria, the figures for all but one of the samples of Table 6 show a fairly good grade of water; certainly not one which is polluted in the accepted sense of the term. The findings for sample No. 17 are not easily explained except on the basis of contamination at the time of taking the sample.

The remarks on gas formation in these samples should be amplified. In no case did gas appear in the presumptive tubes during the first 24 hours of incubation. In the second and

Table 6

Results of Treated Water Sample Analyses, Georgetown, Kentucky, November 18 and 19

Sample Station Number	Total Count at 37° per cc.	MPN of coliforms per 100 cc. confirmed	Remarks
a. Samples of November 18 [x]			
1	600 [z]	Less than 2.2	No gas formation.
2	25	" " 2.2	48 hr. gas formation in three of five 10 cc. presumptive tubes not confirming.
3	5	" " 2.2	" " " " " one " " " " " " " "
4	25	" " 2.2	" " " " " two " " " " " " " "
5	10	" " 2.2	" " " " " four " " " " " " " "
6	5	" " 2.2	" " " " " four " " " " " " " "
7	10	" " 2.2	" " " " " all " " " " " " " "
b. Samples of November 19 [x]			
3	10	Less than 2.2	48 hr. gas formation in three of five 10 cc. presumptive tubes not confirming.
8	10	" " 2.2	No gas formation.
9	800 [z]	" " 2.2	" " "
10	40	" " 2.2	48 hr. gas formation in two of five 10 cc. presumptive tubes not confirming.
11	5	" " 2.2	No gas formation.
12	10	" " 2.2	" " "
c. Samples of November 19. (Specially studied) [y]			
13	160	Less than 1	48 hr. gas formation in seven of ten 10 cc. presumptive tubes not confirming.
14	20	1	" " " " " six " " " " " " " " (One tube partially confirmed)
15	10	Less than 1	48 hr. gas formation in one of ten 10 cc. presumptive tubes not confirming.
16	25	1	" " " " " four " " " " " " " "
17	10	16	" " " " " ten " " " " " " , 7 confirmed.
18	20	Less than 1	" " " " " nine " " " " " " not confirming.

(x) Five 10 cc. lactose broth tubes used for presumptive test. Partial confirmation by 2% Brilliant Green Bile broth and MacConkey's agar.
(y) Ten 10 cc. lactose broth tubes used for presumptive test. Partial confirmation as above.
(z) Estimated number.

third samples of Table 6a it showed in relatively small amounts; in the fourth, fifth and sixth samples one tube of each set showed explosive gas formation. Fewer samples of Table 6b tended to show gas but here again one tube of each set showed explosive development. The general distribution of gas formation among the dead-end samples of Table 6c more nearly resembles that for those taken on the main grid the previous day (Table 6a), and here also it was explosive in about the same ratio.

Detailed attention has been given to these findings on late gas-formation because they represent a common finding in connection with gastro-enteritis outbreaks and suggest the presence of anaerobes which may not be present in large numbers but nevertheless may be etiologically related. Apparently the use of anaerobic culture methods on samples of water suspected of causing gastro-enteritis is basically indicated but was, unfortunately not resorted to in the present study.

The samples of Table 6c were studied in more detail than can be shown there. Tubes showing no gas were streaked on plates of MacConkey's medium and suspicious colonies fished for further study. In addition two 100 cc. portions of each sample were filtered by suction through discs of diatomaceous earth in Jenkins filters, the two discs being planted in tubes of lactose broth and Selenite F media, respectively, for enrichment. After 24-48 hours the tubes were streaked on MacConkey's plates and suspicious colonies again fished.

By these methods five of the six samples were found to contain bacteria of the genus Pseudomonas. One sample contained this organism alone, the other four contained also bacteria of the genus Alkaligenes. The one sample from which no cultures could be obtained came from a dead-end at a C.C.C. camp nearly a mile out of town where no cases occurred, despite the use of city water.

The study of individual patients gave no further clue to the etiology. The symptomatology has been briefly outlined above. Fever was slight or absent. One blood culture was secured and proved sterile. The blood of six patients was studied microscopically: five showed white blood cell counts ranging from 5,000 to 7,000 and one showed a white count of 12,500. The differential count was uniformly within normal limits.

Six stools freshly passed, were studied microscopically: three showed considerable numbers of a small yellow cell not much larger than a red cell but oval in outline and not dissolving in acetic acid. Presumably these were small yeast cells. The remainder showed no abnormality.

Twenty stools were examined bacteriologically within three hours of passage, on the following media; MacConkey's agar, Desoxycholate citrate agar, Salmonella-Shigella agar and Bismuth-Sulphite agar. In addition some of each stool was inoculated into Selenite F enrichment medium and subsequently streaked on MacConkey's agar. Eighteen of the twenty showed a rather sparse growth of the usual coliform colonies. One showed a nearly pure culture of *Pseudomonas* and one showed colonies which gave the cultural reactions of a *Salmonella*. Through the courtesy of Dr. P. R. Edwards of the University of Kentucky, Lexington, this organism was finally identified as *Salmonella panama*. Of the eighteen stool specimens showing the usual coliform flora seven also showed other types of colonies. These, on further study, were found to be coliforms in five instances, *Pseudomonas* in two and *Proteus* in two. One stool containing *Proteus* and one containing *Pseudomonas* also had colonies later found to be coliforms.

Only one organisms, *Pseudomonas*, was obtained from both the water and the stool samples and this was found inconstantly in the latter. Despite the inconstancy of its appearance in the stools it seemed advisable to study this organism in some detail. It was formerly regarded as one of the causes of intestinal tract infection; it is known to produce a rather complex toxin fatal to experimental animals when injected subcutaneously. Lacorte, in Brazil, recently described diarrheal death in animals when comparatively small doses of culture filtrates are injected into the duodenum. It is known, also, that the organism can produce cyanide in small amounts of culture. There has recently been a recrudescence of interest in this group as one of the causes of diarrheal disease, if not of gastro-enteritis.

Studies in our laboratory, which have not as yet been completed, do not, however, support the view that either filtrates of lactose broth cultures or the cultures themselves when taken by human beings orally can produce any symptoms whatever. As many as four billion organisms have been taken of one strain with no appreciable effect. When large numbers are ingested the organism may appear for a day or so in pure culture in the stool but it is quickly displaced by the normal intestinal flora.

Some importance nevertheless may be attached to *Pseudomonas*. There is evidence that this organism can inhibit or mask the growth of coliforms in mixed cultures, raising the question of whether the absence of gas in presumptive tests where this organism is found definitely excludes the possibility of coliforms also having been present in the sample.

The failure of the laboratory to find a responsible agent in water implicated, for the reasons outlined in the preceding section, was not wholly without precedent. The conditions which cause water-borne gastro-enteritis have never been satisfactorily demonstrated. Reasons have been given, however, for considering that the condition has an infectious, probably bacterial, basis.

General Observations.

Back-siphonage and cross connection possibilities were explored by Mr. Perkins of the Kentucky State Health Department. There are no local industries active in Georgetown, the local pasteurizing plant and the college being probably the largest users of water in the city. Certain defective installations were uncovered but it was difficult to see how these could account for so wide-spread an outbreak as the one in question.

It seems more likely that what was probably an infection came through the plant from the original source of supply, the Royal Spring.

Reasons have been given, in the report presented shortly after the outbreak, for considering the treatment of water at Georgetown as inadequate. Filtration, and at times chlorination, was shown to be faulty and this is corroborated by the periodic high total counts shown in Table 5.

When fires occur, and there were an unusual number of these during the preceding week,* the water sometimes receives rather hasty treatment owing to lack of adequate water storage in the distribution system. At such times also the flow in the mains is accelerated and sedimented material and bacteria in the mains may be stirred up in considerable numbers.

The possible sources of pollution of the spring were therefore explored at some length.

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* Four fires occurred on the following dates: Wednesday, November 13, at noon; Saturday, November 16 at 5 a.m. with another at noon; Sunday, November 17 at 9 a.m.

The Royal Spring is really the outlet of a small underground river flowing through cavernous limestone formation until it reaches the surface at the bottom of a small limestone cliff near the center of Georgetown. There are several such streams in the vicinity of Georgetown, each draining, usually, the higher ground southward from the outlet. Surface water reaches the underground collecting caverns through "sink-holes" in the bottom of "sinks" -- more or less circular depressions from a few feet to several hundred yards in circumference -- scattered along the course of the underground streams.

The practice prevails of dumping all manner of debris in the "sink-holes" down which surface water drains from these depressions into the underground streams. Such accumulations gradually disappear - even when piled high. There is a small "sink" nearly filled with ashes located within a hundred feet of the spring. On farms further from the city, "sink-holes" are convenient burial places for dead animals. The carcasses of the larger animals are, in general, sold to a rendering plant. Sewage also may be piped off to a "sink-hole" to avoid the necessity of building a cess-pool. Many sink-holes are to be found in pastures south of Georgetown where there are extensive accumulations of animal feces.

Under these conditions it is not hard to see why rains over the collecting surface of the spring vastly - and sometimes with incredible rapidity - increase the bacterial load of the water issuing from the Royal Spring. In the underground caverns there are doubtless sludge deposits and the increased current after rains probably stirs these up much as flushing a hydrant roils a water main.

With conditions such as this constant vigilance by trained operators and faultless equipment for treating water are indicated. These are not available in Georgetown.

Summary.

An explosive epidemic of gastro-enteritis, of short duration, occurred in Georgetown, Kentucky, on November 16, 17 and 18, 1940, following rains that caused increased flow and turbidity of the water from Royal Spring, the source of the city's water supply.

Bacteriological records showed that the effectiveness of water filtration and/or chlorination was quite variable.

The incidence among persons using private well and cistern water for drinking was much lower than in persons using the city supply.

Age and sex distribution of cases in a canvassed population was nearly uniform.

There was a definite incubation period of 36-72 hours, suggesting an infectious, rather than a toxic, etiology.

Very few confirmed coliforms were found in samples taken during this investigation. This might be due to increased chlorination beginning when the outbreak became apparent. It is, however, possible some organisms, not ordinarily detected by standard bacteriological methods, may be the cause of attacks of the type of gastro-enteritis encountered in this outbreak.

Acknowledgment.

Acknowledgment is made of extensive courtesy and assistance on the part of the Kentucky State Health Department, the Scott County Health Department and the officials of the local water plant, also Dr. P. R. Edwards of the University of Kentucky for assistance in determining the identity of certain organisms.

APPENDIX III.

Gastro-enteritis Outbreak in Navarre, Ohio

EPIDEMIOLOGICAL STUDY OF WATER-BORNE GASTRO-ENTERITIS IN NAVARRE, OHIO

By
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On the 23rd of February, 1941, an unusually high prevalence of gastro-enteritis in the village of Navarre, Ohio, was brought to the attention of Dr. Underwood, of the Stark County Board of Health. The County Health Department obtained four water samples from scattered points on the distribution system which, upon bacteriological analysis at the State Health Department laboratory, revealed the presence of coliforms. Examination for coliforms, presumptive and confirmed, were made on two 10 cc. and two 1 cc. portions of each sample. The results of these analyses are shown in Table 1.

TABLE 1

GAS FORMATION, CONFIRMED FOR COLIFORMS, IN FOUR TAP WATER SAMPLES FROM NAVARRE, OHIO
FEBRUARY 24, 1941

Sampling Point	Volume of Portion			
	10 cc.	10 cc.	1 cc.	0.1 cc.
High School	+	-	-	-
"Big Wick's Place"	+	-	-	-
Navarre Club	+	+	+	-
Home of D.A.Fisher	+	+	+	-

On the 25th a routine sample was taken independently at the school and showed no coliforms. On March 1st the State Health Department, in cooperation with the Stark County Health Department, made a careful study of the local situation and took duplicate samples from (1) water at the pumping station before the pumps were started, (2) the same after the pumps had been in operation for a considerable period, (3) the Sohio Gas Station, (4) the Herwick Saloon, (5) Reamer's Filling Station, and (6) the High School. Each duplicate sample was examined independently, making twelve samples for this one day. Those from the High School both showed gas confirming for coliforms in one of the two 10 cc. portions - none in the 1 cc. portions. The remaining samples were negative for coliforms.

An emergency hypo-chlorinator was obtained and installed and on March 8 duplicate samples from three points on the distribution system were all negative.

Through the cooperation of the State and County Health Departments the Gastro-enteritis Study Unit of the Ohio River Pollution Survey was permitted to make an extended study of the outbreak, beginning on the 7th of March. A house-to-house canvass of a considerable portion of the village population was first made and then a series of field laboratory and sanitary engineering tests were conducted.

Results of House-to-house Canvass.

The village of Navarre had 1703 inhabitants at the time of the 1940 census. The canvass included 382 persons in 106 households distributed throughout the village. A total of 115 cases occurred in the canvassed population. The incidence of 30 per cent obtained from these figures is low for reasons which will be stated below.

Data on the time of occurrence of cases are somewhat obscure. The canvass was begun on March 7 and the outbreak had occurred fully two weeks before that time, so that dates of onset were not readily obtained, though a calendar was carried by the canvassers to assist the informants when there was doubt. Table 2 gives, however, the best available picture of the distribution of cases in respect to time.

TABLE 2

DATES OF ONSET OF CASES IN THE CANVASSED POPULATION

Cases	Dates of onset February							
	Prior to Feb. 22	22 Sat.	23 Sun.	24 Mon.	25 Tues.	26 Wed.	27 Thurs.	28 Fri.
Cases with onset on given date	7	20	26	13	8	4	3	2

There were some definite irregularities about the distribution of cases within the village. The Northern, Eastern, and Central portions suffered heavily but the Western and Southern portions had few cases.

The age-incidence shows no striking differences in the various decades. The figures for the canvassed population are given in Table 3. The disorder tended to prevail in the middle decades of life and was considerably less in the younger and older decades.

TABLE 3

AGE-INCIDENCE OF GASTRO-ENTERITIS IN THE CANVASSED POPULATION OF NAVARRE, OHIO

Age	Attacked	Total Canvassed	Percent Attacked
Under 2	2	11	18
2 - 9	14	55	25
10 - 19	21	61	34
20 - 29	21	55	38
30 - 39	20	50	40
40 - 49	15	54	28
50 - 59	12	45	27
60 and over	8	49	16
Unknown	2	2	
All ages	115	382	30

This age-incidence is quite characteristic of both person-to-person contact outbreaks and of water-borne outbreaks. Against the contact hypothesis is the explosiveness and short duration of the outbreak as shown in Table 2. Influenza itself could hardly have reached a peak and declined so rapidly as this outbreak did. More definite, however, than the foregoing indication is the difference between the incidence among persons drinking water from private well supplies and those drinking water supplied by the city. Fifty-one persons were found using private wells of whom only 7, or 13 percent, were attacked. On inquiry it was found that every one of these 7 had habitually used village water while at school or at work. All of the individuals sure of having taken no water from the public supply escaped. Most of these persons had had the same contact opportunities as others living in the village.

Of the 331 canvassed individuals using the village supply habitually, 108 were attacked - an incidence of 33 per cent. Had the canvass been made closer to the time of the outbreak more cases might have been recalled. It was noted that inquiries made in the later days of our investigation showed fewer cases than those made in the earlier days, even in the same parts of the village, suggesting that mild cases had been forgotten. It is probably safe to put the figure for the village as a whole at not far from 40 per cent.

The relative immunity of the Western and Southern sides of the village is not difficult to explain. These are areas of relatively low water use and of correspondingly little flow of water through the mains, the principal flow being rather from the pumping station and storage tank in the Northwest to the dairy and bakery towards the center of town and the Southeast.

No other common factor could be found. Food was bought at various stores. Baked goods were supplied as often by the local bakery as by a delivery wagon selling goods made in a distant city. The incidence was about equal among families on several milk routes.

As in other places where these outbreaks have occurred, many statements were collected during the canvass to the effect that a number of other more or less distant localities had been simultaneously affected, and that persons on private well supplies both in the village and on outlying arms had been attacked. These reports were carefully investigated. In one instance, what appears to have been a typical attack (but was possibly a case of food-poisoning) was found on a farm near the village limits where the use of village water was denied. In other instances isolated cases were found on the surrounding farms; invariably among children attending the village school. The fact that these cases did not spread to others in the household is further evidence against the hypothesis of spread by person-to-person contact.

These outlying cases are of some epidemiological importance in addition to the assistance they offer in determining the vehicle for the outbreak, for it will often be found that they supply sufficient data for establishing an incubation period. The schedule used in canvassing provides a space for recording visits away from home at the time of the outbreak and visitors to households in Navarre from out of town during the same period. Only two such visitors to a canvassed household in Navarre could be traced; they were a wife and her husband who had come to Navarre from Cuyahoga Falls to visit the wife's family. The two had remained for only an hour; neither eating any food and only the wife drinking water. Two days later the wife was attacked with nausea, cramps and diarrhea. The incubation period of 36 to 72 hours has been noted in other outbreaks and offers a strong clue to the fact that a true infection and not a mysterious "poison" underlies the syndrome of water-borne gastroenteritis. It was possible to learn of only two attacked persons who left town during the outbreak. Both were school teachers who left Friday evening and were sick immediately on their return Monday morning. The probability is that they had been infected on Friday because the incubation period would otherwise be too short. Their evidence would place the infectious element in the water as late as Friday the 21st.

Laboratory and Sanitary Data

The laboratory evidence indicating that pollution of the water supply had occurred has been presented in the introduction. It remained to determine whether this pollution had occurred at the source of supply or at some point along the distribution system. As the outbreak seemed too widespread to have resulted from a back-siphonage or defective cross connection, attention at first centered on the source of supply.

A detailed report of the sanitary study of the wells and pumping station of Navarre made by Mr. Lathrop on March 1, 1941, is on file in the Sanitary Engineering Division of the Ohio State Health Department. Here it suffices to note that five wells in the Northwest part of the village, from 30 to 40 feet deep and with casings about twenty years old, constituted the water supply. They form a line parallel with an old canal (now a relatively stagnant lagoon) and about forty feet from it. The remains of the canal tow path lie between the canal and the wells. On the other side of the line of wells and from 50 to 100 feet away flows the Tuscarawas River in a sharp curve. When the wells were first installed they must have been further from the river than at present, but now the current has undercut the bank so that it comes within fifty feet of one of the wells. When the river is in flood the well-heads are submerged; only a two foot rise above the level observed at the time of the survey would have accomplished this. There had been, however, no such flooding of the well-heads for months before the present outbreak.

When uncapped, these wells flow a few inches over the casing - indicating a head of water greater than the river and perhaps greater than that of the nearby canal. Continuous pumping, however, draws the level down 10 or 12 feet.

The pumping station on the line of wells contains an electrically driven pump which sends the water to the main on Wooster Street and into the village grid. An elevated storage tank provides pressure when the pumps are not working and is located on the North edge of the town at the head of Park Street.

Only one of the wells (Number 5) could be tested individually because the valves at the well-heads were considered rusty and unreliable. This well, however, was the one nearest to a badly polluted section of the lagoon and also nearest to the river. Tests were accordingly first made on this one well on March 21. The bacteriological tests consisted of a standard water analysis for coliforms, with detailed attention to the type of growth in the lactose broth tubes. Two supplementary chemical tests were also made - for chlorides and for hydrogen ion concentration. The characteristics of the two potential sources of pollution - the river and the lagoon - with respect to the various tests may be briefly outlined.

The sewage from the city of Massillon and various industrial plant wastes are carried by the river, which therefore was high in coliforms and chlorides but relatively low in pH (7.1). The lagoon showed few coliforms (about 20 per 100 cc.), the chlorides 8.5 p.p.m. and the pH was 7.7. Any coliforms in the wells could come from either source and, as the normal well water customarily ran lower in chlorides than the river or the lagoon, the chlorides should rise with prolonged pumping if pollution were drawn in from either source. The hydrogen ion concentration of the wells might, however, assist in differentiating. The normal well water ran a pH of 7.3 - 7.4, and a tendency for this to drop or rise under prolonged pumping would indicate pollution from the river or the lagoon, respectively.

The results of the tests on well Number 5 are given in Table 4 under date of March 20. The continued absence not only of coliforms but of any growth whatever in the fermentation tubes, the tendency for the chlorides to drop, and the relative constancy of the pH gave a fairly definite proof of the lack of pollution of the well from either source during the hour and a half test run. Eleven and a half inches of vacuum were carried during this period which was considered excessive for prolonged pumping. This well was, therefore, cut out for the night and allowed to refill. Pumping on it was resumed in the morning for another hour and a half. Two samples were secured from this run - an early and a final one. The results of tests on the second run confirmed the first; they are given in Table 4 under date of March 21.

TABLE 4

TEST OF WELL NO. 5, NAVARRE, OHIO

March 20 and 21, 1941

Date	Time	Total Count per cc.	Coliforms Per 100 cc.	Chlorides p.p.m.	Hydrogen Ion Conc.
March 20	3:30 P.M.	3	0	4.7	7.4
	3:50 P.M.	2	0	4.0	7.4
	4:10 P.M.	1	0	4.0	7.4
	4:25 P.M.	0	0	3.5	7.4
	5:15 P.M.	1	0	3.7	7.4
March 21	9:45 A.M.	0	0	5	7.2
	11:36 A.M.	0	0	-	-
	10:40 A.M.	0	0	4.7	7.3

As the remaining wells could not be tested individually it was decided to make a prolonged pumping test on all the wells and to waste through hydrants at dead ends in order to prevent excessive pressures in the system. An order was issued to boil all drinking water in the village on the morning of the test, a pressure recording gauge was installed in a home near the uppermost part of the system, and the elevated tank filled and cut off to provide water for use in case of fire. The emergency chlorinator was then shut off and the pumps were started at 10 a.m. Hydrants were successively opened until six were flowing at one time. It was found possible to maintain fair pressure in the system under these conditions, but the vacuum at the wells reached 12 inches - the maximum it was felt the wells could take, and at 1:30 p.m. the pumping and wasting were discontinued.

During this period regular samples were taken at the wells for bacteriological and chemical tests as on the test of well Number 5. Samples were also secured of the turbid water shortly after dead end hydrants were opened and also just before they were closed. A pronounced hydrogen sulphide odor was noted in the water at the pump-house and at several of the dead ends toward the close of the test period.

Table 5 shows the results of the various tests at the wells. Table 6 shows the findings for the samples from the dead ends, both initially and at the end of the run.

TABLE 5.
BACTERIOLOGICAL AND CHEMICAL FINDINGS
DURING TEST OF WELLS,
NAVARRE, OHIO
March 25, 1941

Sample No.	Time	M.P.N. Coliforms per 100 cc.	M.P.N. Bacteria per 100 cc.	Slow Lactose Fermenters	Pseudomonas	Chlorides p.p.m.	Hydrogen Ion Conc.
1	10:05 A.M.	0	2.2	0	0	7.5	7.3
2	10:35 A.M.	0	0	0	0	4.0	7.3
3	10:50 A.M.	0	0	0	0	4.0	7.3
4	11:19 A.M.	0	0	0	0	4.2	7.3
5	11:50 A.M.	0	5	0	0	4.0	7.3
6	12:20 P.M.	0	9	0	+	4.0	7.3
7*	12:50 P.M.	0	12	0	+	4.0	7.3
8*	1:20 P.M.	0	39	0	+	4.0	7.3

* Strong odor of hydrogen sulphide in these last samples.

The most striking fact about Table 5 is that an increasing number of bacteria begin to appear after prolonged pumping as shown in the column headed "M.P.N. per 100 cc." from 11:50 a.m. to 1:20 p.m. These bacteria were of two kinds only: One, a Pseudomonas producing a characteristic water-soluble green pigment on certain media and the other an organism as yet unidentified. The latter appears to be only a saprophyte, but the former may conceivably have pathogenic significance. Organisms of the genus Pseudomonas have long been suspected of causing enteric disturbances. However, several detailed studies have been made on them in this laboratory, whole cultures being ingested by volunteers without evident ill effects. Their appearance in water may, however, be of significance from other points of view. It is known that they are very widely distributed in nature and that they are common in sewage. They are more filterable than coliforms and conceivably might be an early index to pollution. Organisms of this genus are known to produce cyanides under certain conditions of growth. Finally, their presence may interfere with the detection of coliform organisms.

These facts would seem to warrant making an effort to rid the supply of these organisms even though their direct pathogenicity cannot be proven. Because they occur, even after prolonged pumping, in small numbers, it is probable that they originate from one well only. This well may also be the source of the hydrogen sulphide odor noted as being particularly strong at the very end of pumping. Because of the difficulty of determining which well is involved, it may be necessary to rely solely upon chlorination of the supply as the most practical way of dealing with these organisms. In this connection, however, it should be noted that the occurrence of hydrogen sulphide is an unfavorable factor where chlorine is used, tending to limit considerably its effectiveness, unless proper allowance is made for absorption of chlorine by the sulphide.

So much for the findings of the study of the source of supply. The results of the study of the distribution system are presented in Table 6. The total count per cc. is seen to have been abnormally high only in the Ohio Street Loop, suggesting some degree of stagnation. Frequent flushing of this loop would seem to be indicated. The index type of coliforms was not encountered in the test at any time. The bacterial M.P.N. was high due to the extensive stirring up of the system. Late lactose fermenters and Pseudomonas were isolated from all but a few of the samples.

TABLE 6

BACTERIOLOGICAL FINDINGS
ON SAMPLES FROM DEAD-ENDS AND HYDRANTS
NAVARRE, OHIO

Sampling Point	Total count per cc.		M.P.N. Coliforms per 100 cc.		M.P.N. Bacteria per 100 cc.		Slow Lactose Fermenters		Pseudomonas	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed
North & Main Sts.	2	0	0	0	Over 110	21	+	+	+	0
N.Market & Corp. Line	1	0	0	0	Over 110	9	+	+	0	+
4th & Columbiana Sts.	1	0	0	0	Over 110	39	0	0	0	+
Columbiana & Canal	2	0	0	0	Over 110	39	+	+	0	+
Tuscarawas & 4th Sts.	0	1	0	0	Over 110	39	+	+	+	0
Tuscarawas & 2nd Sts.	1	0	0	0	Over 110	39	0	+	+	+
Ohio and Main Sts.	50	0	0	0	Over 110	39	+	+	+	0
Ohio & Wilmott Sts.	59	1	0	0	Over 110	15	+	0	+	+
Wooster & E.Corp.Line	0	0	0	0	2.2	39	0	+	0	+
Canal & Main Sts.	1	0	0	0	48	39	+	+	+	0

While no evidence was found of pollution from points inside the water distribution system on this rather drastic test, there are grounds for believing that such pollution actually may have taken place. The coliforms found in the early samples examined by the State Health Department apparently could not have come from the wells, and if they did not they must have come from some point inside the system. The peculiar distribution of cases in the village offers some rather uncertain clues as to the point of entrance. Cases were most numerous (nearly 100 per cent) on the line from the elevated storage tank at the head of Park Street down to Wooster Street and along Wooster Street toward the center of town. The west end of Wooster Street is supplied by the main carrying water from the pumping station to the elevated storage tank and few, if any, bona fide cases occurred on this main. The inference is that pollution occurred at some point on Park Street north of Wooster and that polluted water either filled the tank, whence it was carried throughout the village, or that it was fed into the main close to the base of the tank and so was distributed to the town. With the exception of a plugged sewer connection for a house nearly at the top of the hill on Park Street, no remotely conceivable source for pollution was found at the time these studies were made. However, the distribution of cases makes this a distinct possibility.

The maintenance of a fair chlorine residual in the water at all times would tend to minimize the danger of recurrence if this hypothesis is correct and would also serve to help to remedy the situation noted under the discussion of the bacteria found in the water at the wells. The advisability of safeguarding this supply by continuous and effective chlorination cannot be too strongly recommended.

Periodic sampling of the water at various points throughout the distribution system, including two or three samples from homes near the tank, is also recommended, on the chance that a mild recurrence may be detected.

A pressure recording gauge located either at the pumphouse or at the top of the hill near the storage tank would provide invaluable information about conditions within the system and about the frequency and duration of pump runs. Lack of such data greatly complicates the understanding and prevention of outbreaks like the present one.

Grateful acknowledgment is made of the active cooperation in this study rendered by the Stark County Board of Health and by officers of the State Department of Health.

APPENDIX IV.

Dysentery Outbreak (Shiga) in Adair County, Kentucky

LABORATORY FINDINGS IN AN OUTBREAK OF SHIGA DYSENTERY

By
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On June 26, 1941, the Kentucky State Health Department requested the services of the mobile laboratory of the Epidemiological Unit of the Ohio River Pollution Survey for the study of an epidemic of diarrhea with an unusually high mortality in Columbia, Adair County, Kentucky. The general epidemiological findings of this outbreak are to be described by Caudill and others elsewhere; the present note will outline some of the advantages of having a well-equipped mobile laboratory for use in epidemiological control of diarrheal disease outbreaks in areas remote from centers with laboratory facilities and will give certain basic laboratory findings in detail.

The mobile laboratory used in the study here described was housed in an 18-foot trailer towed by a coupe. Electricity for lights, hot plate, incubator, oven, and serological bath was provided through an electric cable with sharp pointed spring clamps for attachment to any electric service wires carrying sufficient amperage and voltage. Water was obtained by a hundred foot hose attachable to any faucet. Tanked gas was carried for the autoclave and for Bunsen burners. When the trailer was located, the tow car could be unhitched and driven independently for the purpose of distributing specimen containers and bringing specimens back to the laboratory.

This unit was based at Cincinnati, Ohio, some two hundred miles north of Columbia. On the day that the request for the laboratory was received a stock of MacConkey's and of Krumwiede's triple sugar agar was made up and other media of possible value in the isolation of organisms of the Eberthella, Shigella and Salmonella groups from stools were stocked on the trailer in dehydrated form.

The laboratory left Cincinnati at 7:30 a.m. on June 27, arrived in Columbia at 3:30 p.m. and was located and set up, ready for operation, by 4:00 p.m. of the same day.

The choice of media was not a hard one to make when a few cases had been reviewed hastily. Patients were of all ages and appeared to be toxic, stools were passed frequently and consisted of limited amounts of tenacious pus and mucus, or of blood-streaked creamy pus and mucus. MacConkey's agar and Shigella-Salmonella agar (Difco) plates were poured and prepared for streaking. All plates were cooled under wet towels in a stream of air from an electric fan.

Meantime, stool specimen outfits were left with a number of patients. Each outfit consisted of paper plates, paper spoons and sterile metal containers without preservative. The patients were instructed to defecate on the plate and transfer the specimen to the container by means of the spoon. Plates were left in the homes upside-down on a table and covering the spoons so that flies could not contaminate them. Where freshly removed diapers were available, these were taken to the laboratory direct, without using the containers.

By 8:00 p.m. - four hours after the trailer was installed - the plates had dehydrated sufficiently for use and eighteen stool specimens had been secured. These were inoculated in duplicate, both on MacConkey's and on Shigella-Salmonella agar and were incubated at 37°C. By noon of the following day a fair growth of colonies, suspicious in appearance, was present on many of the plates. Presumptive agglutinations were made directly from the colonies on the plates with 1/20 dilutions of the following diagnostic rabbit antisera: Shiga, Polyvalent dysentery and Flexner. The majority of the colonies selected gave good agglutination with the first two, but none with the last antiserum. Suspicious colonies were also fished to Krumwiede's triple sugar agar slants and positives subsequently were purified and carried through the various cultural and serological reactions necessary for identification.

One of the outstanding advantages of the use of mobile laboratories in the control of epidemics like the one described here is that quick and efficient laboratory diagnosis is supplied. Although further stools were examined upon which more comprehensive data will be presented below, it may be of interest to outline briefly the final results of the analysis of the first eighteen stool specimens to illustrate this point.

When carried through triple sugar, sugar fermentation, and serological reactions, twelve of the eighteen stool specimens were found to be positive for Shiga dysentery. The eighteen specimens had been secured from fourteen patients (some patients having supplied more than one stool) and the number of patients represented by the twelve positive specimens was eight. The six negative sets of plates were from the stools of five patients all of whom were in the second or third week of their illness; one set of plates was overgrown and could not be studied further.

Having completed the first, or diagnostic phase of the work the mobile laboratory was brought back to Cincinnati, on June 30, for administrative reasons. On July 21, the trailer was returned to Columbia where it remained until August 8. In the interval the epidemic in Columbia proper had begun to subside, but small rural foci of dysentery had developed, some of which were small unrelated outbreaks perhaps of Flexner and paradysentery, others of which were clearly related to the urban focus of Shiga. The aid of the Red Cross had been invoked and a small hospital for dysentery patients had been set up. Also, the question of the advisability of a convalescent carrier study had been raised. The second visit of the mobile laboratory had, therefore, not only a case diagnostic, but also a carrier, study function to perform.

As the laboratory was not present throughout the period of the epidemic, it will be evident that the cases diagnosed definitely by stool analysis represented only a limited number of the total cases which occurred during the whole period of the outbreak, including no cases for the period of June 30 to July 21, inclusive. Some attempt was made to bridge this gap by securing blood samples for agglutination from persons who recovered, were in the third or fourth weeks from the onset of their illness, and whohad not been otherwise studied during the acute phases of the attack. Because the carrier study was added to the diagnostic study during the second visit the total number of positive isolations became only a limited fraction of the total number of stool examinations made.

Before presenting the results of the analyses it may be useful to detail the procedure adopted in order to convey some idea of the scope of the work which it is possible to carry out in a well-equipped mobile laboratory. During the second visit of the trailer an assortment of sugar media was stocked to permit cultural study and complete equipment for serological study was also included. The following routine was then adopted: When a stool specimen was brought in it was streaked on duplicate plates of MacConkey's and Shigella-Salmonella agar and in several instances a microscopic examination of the stool was made, the presence or absence of mucus, pus or blood cells being noted.*

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* These microscopic checks were found to be unexpectedly useful because on certain days stools of many convalescents at the hospital were noted grossly to be flecked with red causing some consternation among the attendants. Microscopically, no blood cells could be seen, and the red flecks were identified as fragments of stewed tomatoes served on the previous day.

After 18 to 24 hours incubation at 37°C. the stool specimen plates were examined and suspicious colonies were fished to Krumwiede's triple sugar agar slants. After 24 hours incubation at 37°C. the Krumwiede's slants were read and those with typical Eberthella-Shigella reactions* were subjected to presumptive agglutination and were purified. Purified cultures were transferred to nutrient agar slants to develop growth for antigen for final serological confirmation and were inoculated into mannitol, Glucose, sucrose, lactose, maltose, rhamnose and tryptone broths (the latter for indole testing). In the fourth 24 hour period the first readings were made on the reactions in the sugar media and final agglutination tests were made. Dilutions of Shiga, Polyvalent dysentery and Flexner rabbit antisera up to 1/3600 were prepared for this purpose. Thus by the fifth day a definite conclusion as to the nature of the organism was obtained.

A total of 211 stool specimens were collected from 94 patients. Forty-six of the specimens from 27 patients were found to be positive for dysentery and dysentery-like organisms. A great many of these cases were examined because of the occurrence of diarrhea even though there was no history of contact with, or proximity to, cases or foci of known Shiga dysentery. There were 132 stools from patients whose relationship, familial or geographic, with known Shiga patients led to a strong suspicion that they were Shiga cases. Of these 31 were positive for Shiga dysentery. The 132 specimens were supplied by 49 patients and the 31 positive specimens represented a total of 17 diagnosed cases of Shiga dysentery. The remaining positive cultures, which were from cases of dysentery, Shiga (2 cases), Sonne (4 cases), Hiss (2 cases), Flexner (1 case) and typhoid fever, E.typhosa (1 case) will be discussed when the cultural and serological characteristics of the organisms are presented.

The low ratio of positive specimens to total examinations was due in part to the fact that a large number of examinations for carriers was made. A better measure of total effectiveness in diagnosis will be given below when the results of the analysis of stools of this group are presented according to the duration of the disease.

As nearly all stool examinations were made in duplicate on MacConkey's agar and on Shigella-Salmonella agar it may be useful to compare the results obtained by the use of the two.

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* Acid butt, without gas and no change in slant.

Table 1

Comparison of MacConkey's Agar and Shigella-Salmonella Agar for the Isolation of Organisms of the Shigella Group from Stools.

Findings on duplicate plates of MacConkey's and Shigella-Salmonella Agar	Number of Stool Specimens
Both plates positive	25
Both plates negative	155
MacC. positive, S.S. negative	3
MacC. negative, S.S. positive	21
Comparison not possible	7
	211

These results are shown in Table 1 where, of 204 examinations in which a comparison is possible, MacConkey's agar gave positives in 28 examinations, and Shigella-Salmonella agar in a considerably higher number - 46. Because of the large number of negative examinations made necessary by the carrier study the total percentages of success with either medium are low; a fairer basis might well be the total proportion of each which was positive with either medium or both. By this measure it will be seen that Shigella-Salmonella agar gave 46 out of the total 49 positive, while MacConkey's agar gave only 28.

In actual practice, the advantage from the use of Shigella-Salmonella agar in trailer laboratories is considerably greater than would appear from abstract figures for the following reasons:

1. It is more readily available for use than MacConkey's because it does not have to be autoclaved before use.

2. Plates may be directly streaked with fairly heavy inocula without dilution or other precautions against overgrowth and only one plate need be used for each stool.

3. The number of suspicious colonies tends to be greater and coliform colonies fewer, giving a far better selection.

The extent to which positive stool findings could be made with either medium singly at varying intervals after the onset of diarrhea is shown in Table 2. Here, as in previous discussions of results, the data are given for all findings and then for cases presumed to have been Shiga dysentery on the basis of contact with previous cases or foci of infection.

Table 2

Number of Stools Examined at Specified Intervals After Onset of the Disease and Number and Percent Positive on MacConkey's and on Shigella-Salmonella Agars.

Interval in Days (incl.)	Total number of Stools Examined	Positive MacConkey	Positive S.S.Agar	Percent Positive MacConkey	Percent Positive S.S.Agar
		(A) All Specimens			
0 - 3	37(1)	10	17	28	46
4 - 7	35	12	19	34	54
8 - 15	51(1)	5	10	10	20
16 - 60	70(1)	0	0	--	--
	193	27	46	14	24
		(B) Specimens from Shiga Contacts			
0 - 3	22(1)	8	12	38	57
4 - 7	18	7	8	39	44
8 - 15	31(1)	4	6	13	19
16 - 60	64(1)	0	0	0	0
	135	19	26	14	19

(1) One stool in each of these intervals is not examined on MacConkey's Agar.

The percentages for positives on Shigella-Salmonella Agar in Table 2, it will be noted, are but little higher than those for MacConkey's, but they tend to be consistently higher in the three groups where positives were obtained. Table 2 also shows that by the end of the second week very few stools were found to be positive and that a sizable group of examinations for the third and succeeding weeks (16-60 days) gave no positives at all. These seventy examinations, however, represent stool studies on only 27 patients. If the carrier rate for Shiga dysentery is actually in the neighborhood of 3 per cent* it could easily happen that this number of patients might have been examined without finding one positive. The results of the somewhat limited number of microscopical examinations are briefly presented. A great many of the stools received from patients early in the disease showed mucus, pus and blood upon gross examination. The same findings, of course, were evident on microscopical examination with significant exceptions heretofore mentioned. Where mucus, pus and blood were in evidence, the stool was commonly found to be positive on bacteriological examination. The comparative findings are shown in Table 3.

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* Kolmer, J.A. and Taft, L. "Clinical Immunology, Biotherapy and Chemotherapy, 1st ed., p.568. W.B.Saunders & Co., 1941.

Table 3

Microscopical and Bacteriological Findings in Stools of 35 Patients Where Microscopical Examination was Done.

Microscopical Findings	Total with Specified Microscopical Findings	Positive Bacteriologically for Dysentery
Negative for mucus, pus and blood	17	2(1)
Mucus and pus only	4	2
Mucus, pus and blood	14	12
	35(2)	18

(1) One of these, a fatal case in an infant, was negative on microscopical and positive for Shiga dysentery. The other was a para-dysentery case.

(2) Eight stools showed parasitic ova, as follows: ascaris - 4; enterobius - 3, dog tapeworm - 1.

The cultural, biochemical and serological characteristics obtained on the organisms isolated are presented in Tables 4 and 5. On the basis of the consensus of the characteristics determined the first 19 cultures in order in these tables are judged to be Shiga strains, the next 4 are considered Sonne types, the next 2 Hiss Y, and the last 2 Flexner and E.typhosa respectively.

(Insert Tables 4 and 5)

Table 4

Cultural Characteristics of Organisms Isolated

Culture No.	Krumwiede Triple Sugar Slants		Motile	Indole	Action on Carbohydrates				Alcohols	
	Slant	Butt			Glucose	Lactose	Sucrose	Maltose	Mannitol	Rhamnose
1	NC	A	-	-	A	-	-	-	-	-
2	NC	A	-	-	A	-	-	-	-	-
4	NC	A	-	-	A	-	-	-	-	-
6	NC	A	-	-	A	-	-	-	-	-
9	NC	A	-	-	A	-	-	-	-	-
10	NC	A	-	-	A	-	-	-	-	-
11	NC	A	-	-	A	-	-	-	-	-
13	NC	A	-	-	A	-	-	-	-	-
14	NC	A	-	-	A	-	-	-	-	-
15	NC	A	-	-	A	-	-	-	-	-
19	NC	A	-	-	A	-	-	-	-	-
20	NC	A	-	-	A	-	-	-	-	-
22	NC	A	-	Tr.	A	-	-	-	-	-
23	NC	A	-	-	A	-	-	-	-	-
25	NC	A	-	-	A	-	-	-	-	-
26	NC	A	-	-	A	-	-	-	-	-
27	NC	A	-	-	A	-	-	-	-	-
29	NC	A	-	-	A	-	-	-	-	-
12	NC	A	-	Tr.	A	-	-	A	A	A
16	NC	A	-	-	A	-	-	A	A	A
17	NC	A	-	-	A	-	-	-	A	A
28	NC	A	-	-	A	-	-	-	A	A
7	NC	A	-	Tr.	A	-	-	-	A	-
18	NC	A	-	Tr.	A	-	-	A	A	-
3	NC	A	-	+	A	-	A	-	A	-
24	NC	A	+	-	A	-	-	A	A	-

Table 5

Serological Characteristics of Organisms Isolated.
Agglutination of Antigens by Following Rabbit Antisera

Antigen No.	Anti-dysenteric Sera					Eberthella typhosa
	Polyvalent	Shiga	Flexner	Sonne	Hiss	
1		1280				
2		1280				
4	1280	1280	-	-	40	
6		1280				
9		1280				
10		1280				
11		1280				
13		1280				
14		1280				
15		1280				
19	1280	1280	-	-	-	
20		1280				
22		1280				
23		1280				
25		1280				
26		1280				
27		1280				
29		1280				
12	-	-	-	640	-	
16	-	-	-	640	-	
17	-	-	-	640	-	
28	40	-	-	1280	-	
7	640	-	160	-	1280	
18	320	-	-	-	320	
3	640	-	640	-	320	
24	80	-	-	-	160	5120

Note the dilutions given indicate the highest titre in which at least a 3+ or better agglutination was obtained. All antigens were made up to a standard density and the full titre of the respective antisera for their homologous antigens was: Shiga, 1-1280; Flexner, 1-640; Sonne, 1-1280; Hiss, 1-1280; E.typhosa, 1-5000.

Certain deviations in the characteristics found for the organisms thus listed, from the usual descriptions given in the literature, should be noted. While indole production was not shown by any of the cultures when the test was made by the Ehrlich-Böhme procedure, faint traces (more definite with 7 and 18) of indole were produced by cultures 7, 12, 18 and 22, when tested by the more sensitive method of Gore. According to available descriptions indole is not produced by the Shiga, Sonne and Ambigua members of the dysentery group, nor by E. typhosa, but is produced by the Hiss, Flexner, Strong and Dispar dysentery strains. With the exception of culture 22, which shows a trace of indole produced, all of the cultures considered to be Shiga types, present a very clear-cut picture.

In the instance of the four cultures listed as Sonne types, the serological results definitely support this decision but all four cultures failed to ferment lactose or sucrose with acid production and two of them, 17 and 28, likewise, failed to ferment maltose. Similarly, cultures 7 and 18 are definitely Hiss strains serologically, but strain 18 ferments maltose with acid production.

In general, however, it is thought that the classifications made here are correct and that the clear-cut serological evidence outweighs the minor discrepancies noted in the sugar fermentations. This is supported by the knowledge that the established criteria for this group are not exact. It is only in the case of culture 3, tentatively identified as a Flexner type, that some doubt exists. Its failure to ferment maltose and its agglutination to a fair titre, 1-320, with Hiss antisera suggests a close relationship to the Hiss strain. However, this culture 3 ferments sucrose and the typical Hiss strain does not.

In passing it might be noted that the polyvalent antiserum used by us was remarkably lacking in Sonne antibodies.

An attempt was made to get blood samples for agglutination tests from individuals who were in the third or fourth week from the onset of their dysenteric infection. For various reasons it was found to be difficult to obtain these specimens. Moreover, the quantity of blood secured in each instance was usually small. As a result only 24 specimens of patients' sera were available for study and the volume of each serum was such that they could be tested against not more than four antigens. A representative Shiga strain from the current study (Adair Shiga) and stock dysentery cultures of Shiga, Flexner and Sonne types were selected for use as antigens. Three of the blood specimens obtained, Nos. 2, 13 and 23, were from patients whose stools had been cultured and from which typical Shiga cultures had been isolated. (See cultures Nos. 2, 13 and 23 in Tables 4 and 5). The remaining blood specimens were from individuals who had suffered from dysentery during the current epidemic but no bacteriological examinations had been made of their stools. The results obtained from the study of these patients' sera are presented in Table 6.

Table 6

Results of Agglutination Tests with Patients' Sera* set up against Antigens of Known Species and of a Representative Adair County Shiga Strain

Patient Serum No.	Titre** of Patients' Sera Against Antigens for			
	Stock Shiga	Adair Shiga	Stock y Flexner	Stock Sonne
2	160	320	320	-
13	-	-	160	-
23	20	20	-	-
30	80	80	320	-
31	80	80	40	-
32	-	-	40	0
33	-	20	-	-
34	-	-	-	-
35	-	-	40	-
36	-	-	-	-
37	-	-	320	-
38	-	-	320	-
39	160	320	160	-
40	40	80	320	-
41	20	20	640	-
42	-	-	320	-
43	-	-	-	-
44	-	-	160	-
45	-	-	80	-
46	-	-	20	-
47	-	-	-	-
48	-	1280	320	-
49	640	20	-	-
50	-	-	-	-

* Patients' sera Nos. 2, 13 and 23 were from cases yielding cultures of the same numbers as shown in Tables 4 and 5. All other sera are from uncultured cases.

** Titres recorded here represent a 2+ or better agglutination as set forth on page 235 of Diagnostic Procedures and Reagents, First Edition, 1941, A.P.H.A.

In determining significant titres for agglutination tests consideration must be given to the strength of the antisera used and to agglutinability of the antigens subjected to their action. In obtaining the results of Table 5 fairly potent antisera were used and it is thought that agglutinations below a dilution of 1-320 of the antisera should not be considered significant. With patients' sera, such as those used in obtaining the results shown in Table 6, much lower titres are to be anticipated. Considered from the standpoint of the antigens used the Shiga strain is distinctive serologically while the other members of the dysentery group, particularly the Flexner types, tend toward group, and to some extent auto, agglutination Consequently, it is believed that with patients' sera titres of 1-40 or over against Shiga strains should be considered significant and with the other varieties of dysentery bacteria titres of at least 1-80 and possibly 1-160, should be required.

With these titres as a criterion, it is noted from Table 6 that six patients' sera, Nos. 2, 30, 31, 39, 40 and 49, gave agglutinations to significant titres of 1-40 or higher with the antigen of the stock Shiga culture and six sera, Nos. 2, 30, 31, 39, 40 and 48 yielded significant titres of 1-40 or over with the Adair County Shiga antigen. The failure of serum 48 to agglutinate the stock Shiga antigen while it agglutinated the Adair strain in high titre, and the agglutination by serum 49 of the stock Shiga antigen in high titre while it failed to affect the Adair Shiga antigen above a titre of 1-20 is most unusual. These tests with sera 48 and 49 were repeated twice with identical results. A remarkable specificity in the case of these two sera is indicated. Similarly, the unusually high titres for patients' sera, 1-640 and 1-1280, suggest a marked antibody production for a specific organism. Thus the agglutination of Shiga antigens in significant titres of 1-40 or over by 7 of 24 patients' sera studied is definite evidence, and confirms the observation, that a considerable portion of the dysentery cases in this epidemic were Shiga infections.

No agglutinations of Sonne antigen were observed with any of the 24 patients' sera, even in the 1-20 dilution, although this antigen agglutinated to the full titre of the diagnostic Sonne antiserum used as a control.

Twelve of the 24 patients' sera, namely Nos. 2, 13, 30, 37, 38, 39, 40, 41, 42, 44, 45 and 48, agglutinated Flexner antigen to significant titres. As the bacteriological findings obtained in this study disclosed only one possible Flexner strain this would definitely suggest a very high incidence of endemic Flexner dysentery in this locality with a Shiga infestation superimposed in the present epidemic.

SUMMARY

Using the facilities of a well equipped trailer laboratory a bacteriological study was made of a dysentery epidemic. The marked value of such a mobile laboratory for use at the site of an epidemic is demonstrated.

In this study 211 stools from active cases of dysentery and from convalescents (some as removed as 60 days from onset of infection) were examined.

From these 211 stools representing 94 patients bacteriological diagnosis was made of 27 cases; 19 Shiga, 4 Sonne, 2 Hiss, 1 Flexner, and 1 Typhoid fever.

A brief characterization of the organisms isolated is presented.

A serological study of 24 patients' sera indicates that the current epidemic was a Shiga infection and that dysentery of the Flexner type is endemic in this territory.

Acknowledgments

It is desired to express our indebtedness to various officials of the Kentucky State Department of Health for their co-operation and assistance; also to the various departments of the City of Columbia, particularly the Water Department; and especially to Dr. J. T. Duncan, County Health Officer of Adair County for his cordial and effective co-operation during the time we were making this investigation: and to the medical and nursing staff of the temporary Red Cross hospital that was established at Columbia during the outbreak.

APPENDIX V.

Outbreaks of Gastro-enteritis Traced to Water Supplies

Appendix V

Outbreaks of Gastro-enteritis traced to water supplies.

Locality	First cases noted				Dura. of Epid. (Days)	First Water Samples Taken			Coliforms in early samples	Source of Water Supply	Population		Percent Attacked	REMARKS
		Day of		Day of month		Month	Day	Days from start			Exposed	Attacked		
	Year	Week	Month											
				Source of, and bacteriological evidence for, pollution not found										
Georgetown, Ky.	1940	Fri.	Nov.	15	5	Nov.	18	3	0	Spring	4,500	2,250	50	Unusual no. fires; turbid spring
Cohasset, Mass.	1931	Sun.	Mar.	15	4	Mar.	18	3	0	Wells	3,000	1,000	33	Active flushing of hydrants just before.
Coshocton, O.	1936	Tues.	Feb.	18	N.S.	Feb.	20	2	0	Well	11,000	1,500	17	Freezing & thawing, flooding.
Milwaukee, Wisc.	1938	Mon.	Feb.	7	21+	Daily		0	0	Lake	600,000	- -	11	Sewage blown toward water intake.
				Source of, or bacteriological evidence for, pollution found										
"Indiana Town"	1940	Thurs.	Feb.	1	N.S.	—	—	—	?	River	7,700	- -	26	New filters installed; turbid water in mains.
Navarre, Ohio	1941	Sat.	Feb.	22	3	Feb.	24	2	+	Wells	1,700	680	40	Active flushing of hydrants just before.
Springfield, Mo.	1936		July	16-17	N.S.	July	13-23	0	+	Impounded Spring Well	65,000	20-35,000		Source not described.
"Tennessee Town"	1936	Week ends	June-Aug.	Recurrent	Brief	--	--	—	?	Spring	2,500	765	31	Leak from sewer above spring.
Dallas, Texas	1937		Apr.	8	6	N.S.	N.S.	—	+	Wells	10,000	3,000	30	Source not found.
Altamount, N. Y.	1937	Mon.	May	17	13	N.S.	N.S.	--	+	Surface	900	190	20	Source not found.
Vinton, Iowa	1938	Wed.	Feb.	2	6-7	Feb.	7	5	+	Wells	3,400	2,000	59	Possible pollution of wells.
				Outbreaks with sewage pollution found and followed by typhoid fever cases										
Excursion Steamer	1912		July	29	2-3	--	--	--	N.S.	River	1,200	600	50	Water pumped while steamer over sewer.
Santa Anna, Cal.	1924		Jan.	1	N.S.	--	--	--	+		27,000	10,000	37	Plugged sewer flooding water.
Detroit Mich.	1926		Feb.	25	N.S.	Daily		0	+	River	1,300,000	45,000		Sewage blown toward water intake.
Greater Rostow, U.S.S.R.	1926		Apr.		14-21	Apr.	24	?	+	River	260,000	40,000	15	Leak from sewer above spring.
Chicago Stock Yd. fire	1934		May	19	—	--	—	--	N.S.	Lake	?	300		Cross connection with polluted supply.
Rochester, N. Y.	1940	Thurs.	Dec.	12	4	Dec.	12	0	+	River	280,000	40,000	9	" " " "

Note: N.S. = Not stated.

APPENDIX VI.

D & E Mortality Statistics for 144 Cities of the Ohio River Basin

APPENDIX VI

Diarrhea and Enteritis Mortality Statistics and Related Data for 144 Cities in the Ohio River Basin

City and State	Pop. 1935 (est.)	D. & E. Death Rates per 100,000:1933-37		Altitude feet above sea level	Lat. North	Region	Source of water supply
		Mean	Corrected for Residence				
Decatur, Ala.	15,983	52.5	48.3	568	34.7	South	Surface
Florence	12,329	60.2	57.2	496	34.7	"	"
Huntsville	12,366	59.9	56.3	628	34.8	"	Ground
Cairo, Ill.	12,696	27.2	23.1	311	37.0	N.W.	Surface
Champaign	22,588	8.3	8.5	738	40.1	"	Ground
Danville	38,259	21.7	16.1	601	40.1	"	Surface
Harrisburg	11,531	52.0	58.8	368	37.7	"	"
Mattoon	15,170	18.4	17.7	739	39.5	"	Mixed
Urbana	14,471	17.8	15.1	725	40.1	"	Ground
Anderson, Ind.	44,826	19.2	18.4	873	40.0	"	Mixed
Bedford	13,862	30.5	29.6	697	38.9	"	Surface
Bloomington	21,544	19.7	20.7	748	39.1	"	Mixed
Connersville	12,842	18.7	19.3	836	39.6	"	Ground
Crawfordsville	10,466	18.6	16.9	757	40.0	"	"
Elwood	11,130	46.3	46.3	860	40.2	"	"
Evansville	110,744	22.1	20.3	374	37.9	"	Surface
Frankfort	12,500	17.1	16.9	854	40.2	"	Ground
Huntington	13,130	22.0	22.9	742	40.8	"	"
Indianapolis	389,148	19.9	18.7	765	39.7	"	Mixed
Jeffersonville	12,874	39.3	43.2	448	38.2	"	Ground
Kokomo	34,234	22.2	22.4	814	40.4	"	"
Lafayette	28,119	23.9	17.9	600	40.4	"	"
Logansport	16,945	8.2	7.5	606	40.7	"	Surface
Marion	24,875	25.7	25.2	813	40.5	"	Ground

APPENDIX VI

Diarrhea and Enteritis Mortality Statistics and Related Data for 144 Cities in the Ohio River Basin

City and State	Pop. 1935 (est.)	D. & E. Death Rates per 100,000:1933-37		Altitude feet above sea level	Lat. North	Region	Source of water supply
		Mean	Corrected for Residence				
Muncie, Ind.	51,562	25.4	23.6	942	40.1	N.W.	Mixed
New Albany, Ind.	27,236	21.1	31.9	413	38.3	"	Surface
Newcastle	13,811	21.1	36.3	1,031	39.9	"	Ground
Peru	12,890	7.9	11.8	642	40.7	"	"
Shelbyville	10,667	16.9	24.3	768	39.5	"	"
Terre Haute	61,172	20.7	30.4	485	39.4	"	Surface
Vincennes	17,768	41.5	62.3	427	38.6	"	"
Ashland, Ky.	36,249	54.6	50.2	511	38.4	South	"
Bowling Green	13,448	62.6	65.1	499	37.0	"	"
Covington	69,318	23.8	21.7	513	39.0	"	"
Fort Thomas	9,958	0.0	----	852	39.1	"	"
Frankfort	11,547	32.9	33.9	560	38.2	"	"
Henderson	11,417	66.3	64.3	374	37.8	"	"
Hopkinsville	11,266	69.2	76.8	556	36.9	"	"
Lexington	47,838	43.2	39.7	967	38.0	"	"
Louisville	344,174	20.7	19.9	459	38.2	"	"
Middlesborough	10,896	110.0	91.3	1,066	36.6	"	"
Newport	29,961	6.6	9.9	506	39.1	"	"
Owensboro	25,436	49.6	48.1	403	37.7	"	Ground
Paducah	37,947	55.6	54.5	344	37.0	"	Surface
Jamestown, N. Y.	48,278	8.6	6.9	1,322	42.0	N.E.	Ground
Olean	22,434	16.6	13.8	1,435	42.0	"	Surface
Asheville, N. C.	51,042	29.2	24.2	2,115	35.5	South	"
Alliance, Ohio	23,771	9.5	9.0	1,079	40.9	N.E.	Mixed

APPENDIX VI

Diarrhea and Enteritis Mortality Statistics and Related Data for 144 Cities in the Ohio River Basin

City and State	Pop. 1935 (est.)	D. & E. Death Rates per 100,000:1933-37		Altitude feet above sea level	Lat. North	Region	Source of water supply
		Mean	Corrected for Residence				
Ashland, Ohio	11,894	6.8	6.3	1,077	40.9	N.E.	Surface
Barberton	26,497	10.1	10.1	965	41.0	"	"
Bellaire	12,458	13.2	12.1	677	39.9	"	"
Bucyrus	9,829	14.1	12.1	1,004	40.7	"	"
Cambridge	17,644	14.1	11.7	801	40.0	"	"
Campbell	16,394	7.0	12.0	772	41.0	"	"
Canton	113,816	6.0	5.5	1,050	40.7	"	Ground
Chillicothe	19,599	10.5	10.3	632	39.3	"	"
Cincinnati	476,118	21.3	19.4	554	39.1	N.W.	Surface
Columbus	317,332	15.6	13.7	766	39.9	N.E.	"
Coshocton	10,939	17.[illegible]	16.6	770	40.2	"	Ground
Dayton	225,199	14.5	13.5	764	39.7	N.W.	"
E. Liverpool	24,292	13.8	12.0	686	40.5	N.E.	Surface
Hamilton	57,427	23.4	20.1	598	39.3	N.W.	Ground
Ironton	17,930	48.1	43.3	515	38.5	N.E.	Surface
Lancaster	20,721	18.0	15.7	898	39.7	"	Ground
Mansfield	36,376	8.5	7.7	1,152	40.7	"	"
Marietta	13,865	19.4	16.3	624	39.4	"	Surface
Marion	32,682	16.2	15.4	908	40.5	"	Ground
Martins Ferry	15,969	26.3	18.9	654	40.0	"	"
Massillon	30,877	8.3	7.6	986	40.7	"	"
Middletown	33,190	41.8	37.2	664	39.5	N.W.	"
Newark	32,539	14.9	13.7	822	40.0	N.E.	Surface
New Philadelphia	13,192	6.5	8.0	889	40.4	"	Ground

APPENDIX VI

Diarrhea and Enteritis Mortality Statistics and
Related Data for 144 Cities in the Ohio River Basin

City and State	Pop. 1935 (est.)	D. & E. Death Rates per 100,000:1933-37		Altitude feet above sea level	Lat. North	Region	Source of water supply
		Mean	Corrected for Residence				
Niles, Ohio	17,933	11.1	17.1	887	41.1	N.E.	Ground
Norwood	37,636	1.8	2.8	624	39.1	N.W.	Mixed
Piqua	16,494	12.5	11.4	882	40.1	"	Surface
Portsmouth	47,339	65.1	53.4	497	38.7	N.E.	"
Salem	10,784	10.3	7.0	1,207	40.8	"	Ground
Springfield	72,692	9.7	9.4	983	39.9	N.W.	Surface
Steubenville	38,881	18.6	14.7	711	40.3	N.E.	"
Struthers	11,521	10.5	15.3	834	41.1	"	"
Warren	48,069	6.7	5.2	889	41.2	"	"
Wooster	11,140	12.5	12.0	910	40.8	"	Ground
Xenia	10,713	22.4	24.0	917	39.7	N.W.	"
Youngstown	188,726	9.8	8.8	851	41.0	N.E.	Surface
Zanesville	39,876	19.5	16.2	699	39.9	"	Ground
Aliquippa, Pa.	32,961	10.3	16.0	751	40.6	"	"
Ambridge	23,979	8.1	12.6	750	40.5	"	"
Arnold	10,738	7.4	10.0	786	40.6	"	Surface
Beaver Falls	19,322	11.7	10.9	766	40.7	"	"
Bellevue	10,347	9.7	8.3	727	40.5	"	Ground
Braddock	18,554	13.8	11.0	782	40.3	"	Surface
Bradford	21,197	11.9	11.1	1,437	41.9	"	Mixed
Butler	23,463	8.3	9.9	1,011	40.8	"	Surface
Cannonsburg	13,524	4.8	4.1	933	40.2	"	"
Carnegie	12,988	8.0	11.4	769	40.4	"	"
Charleroi	10,982	0.0	----	764	40.1	"	"

APPENDIX VI

Diarrhea and Enteritis Mortality Statistics and Related Data for 144 Cities in the Ohio River Basin

City and State	Pop. 1935 (est.)	D. & E. Death Rates per 100,000:1933-37		Altitude feet above sea level	Lat. North	Region	Source of water supply
		Mean	Corrected for Residence				
Clairton, Pa.	15,817	10.2	14.9	1,020	40.3	N.E.	Surface
Connellsville	13,031	19.4	15.9	882	40.0	"	"
Coraopolis	10,922	7.3	12.0	720	40.5	"	Ground
Donora	13,789	2.9	4.5	900	40.1	"	Surface
Dormont, Pa.	13,018	1.5	2.1	750	40.3	N.E.	Surface
DuBois	10,549	13.5	10.9	1,399	41.1	"	"
Duquesne	22,591	8.6	13.3	850	40.4	"	Ground
Ellwood City	12,317	13.0	12.0	894	40.9	"	Surface
Farrell	13,742	8.7	13.5	940	41.1	"	"
Franklin	10,075	8.0	9.4	1,000	41.4	"	Mixed
Greensburg	17,248	16.8	12.1	1,100	40.4	"	Surface
Homestead	19,985	12.3	12.9	756	40.3	"	"
Jeannette	17,375	3.8	5.1	1,000	40.3	"	"
Johnstown	66,824	12.0	9.6	1,176	40.3	"	"
Latrobe	10,866	14.7	11.3	1,008	40.3	"	"
McKeesport	58,558	16.3	13.2	752	40.3	"	"
McKees Rocks	18,819	3.4	6.3	725	40.4	"	Ground
Meadville	17,763	12.7	10.3	1,078	41.6	"	"
Monessen	21,317	10.9	13.6	762	40.1	"	Surface
Munhall	13,364	6.0	7.5	754	40.4	"	"
New Castle	50,545	10.4	9.5	806	41.0	"	"
New Kensington	19,152	14.9	12.7	775	40.5	"	"
North Braddock	17,711	4.9	6.6	1,000	40.4	"	"
Oil City	22,476	7.5	6.6	1,027	41.4	"	Ground

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Diarrhea and Enteritis Mortality Statistics and Related Data for 144 Cities in the Ohio River Basin

City and State	Pop. 1935 (est.)	D. & E. Death Rates per 100,000:1933-37		Altitude feet above sea level	Lat. North	Region	Source of water supply
		Mean	Corrected for Residence				
Pittsburgh, Pa.	702,556	8.3	7.2	726	40.4	N.E.	Surface
Sharon	25,614	12.5	10.3	854	41.2	"	"
Swissvale	18,590	5.0	7.6	922	40.4	"	"
Turtle Creek	10,295	3.9	6.7	750	40.4	"	"
Uniontown	21,471	29.8	20.6	1,017	39.9	"	"
Vandergrift	11,089	10.8	15.4	800	40.6	"	Mixed
Warren	15,159	12.1	10.4	1,168	41.8	"	"
Washington	26,080	26.8	21.7	1,039	40.1	"	Surface
Wilkinsburg	32,260	4.1	3.7	922	40.4	"	"
Bristol, Tenn.	13,004	26.1	28.4	1,689	36.6	South	"
Chattanooga	150,751	35.7	32.1	660	35.0	"	"
Johnson City	30,935	25.4	16.0	1,631	36.2	"	Ground
Kingsport	13,156	21.1	16.5	1,270	36.6	"	Surface
Knoxville	119,796	37.8	32.9	905	35.9	"	"
Nashville	171,630	36.7	33.0	498	36.1	"	"
Bluefield, W. Va.	21,371	64.8	40.8	2,560	37.2	"	Ground
Charleston	70,808	84.1	58.9	600	38.3	"	Surface
Clarksburg	29,368	17.6	14.3	1,034	39.2	"	"
Fairmont	25,817	31.2	25.3	886	39.4	"	"
Huntington	88,272	36.6	30.4	565	38.4	"	"
Morgantown	18,220	27.6	28.4	892	39.7	"	Mixed
Moundsville	16,283	7.0	8.9	630	37.4	"	Ground
Parkersburg	34,411	38.8	30.7	601	39.2	"	"
Wheeling	64,385	15.7	12.9	637	40.0	"	Surface